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THE EXPRESSED SAP OF CORN PLANTS AS AN INDICATOR OF NUTRIENT NEEDS[1]

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INTRODUCTION

Ever since it was realized that man has at his disposal materials which may be used to supplement the natural fertility of soils, agronomists have felt the need of reliable indicators of plant nutrient sufficiencies and deficiencies in soils. The hope has always been cherished that the fulfillment of this need would remove much of the difficulty and uncertainty involved in the selection of the proper kind and amount of fertilizer to apply to any given crop and soil.

The search for nutrient deficiency indicators has extended over approximately 100 years, and has yielded many field, laboratory, and greenhouse methods for estimating the needs of the soil for growing crops. Most of these methods, however, with the exception of a few suggested within the last decade, have proved to be of little value. For this reason a review of methods will be confined to brief statements concerning a few of the more recently proposed methods which are popular among investigators at the present time and are being used extensively to diagnose crop needs.

In 1920, Neubauer (*12*)[3] suggested the analysis of rye seedlings which had been grown for 17 days on a sand-and-soil mixture inadequate to maintain optimum growth in a population of approximately 100 seedlings. By keeping the seedlings on a starvation ration, Neubauer believed they would extract from the soil practically all of the available or root-soluble nutrients. Although this method is being used extensively in Germany to detect nutrient deficiencies, considerable disagreement exists as to its practical value. In 1926 Hoffer (*7*) reported a field method for determining the nutrient needs of corn plants by testing the interior stalk tissues chemically for potassium and nitrate needs. These tests are used mainly to confirm certain exterior plant symptoms of malnutrition which Hoffer states are associated with soil deficiencies. A year later Mitscherlich (*11*) revived interest in the pot-test method of Wagner by developing an improved type of pot whose main advantages are its construction of enameled sheet iron and a base pan for collecting the drainage water which may be returned to the soil. The potted soils receive specified fertilizer treatments and are cropped to one or more good indicator plants. The weights of the mature plants are used to determine the fertilizer needs.

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[2] The author is indebted to Dr. W. B. Ellett for permission to use the laboratories of the Department of Agricultural Chemistry in making the chemical analyses; to H. H. Hill, associate chemist, for the nitrate determinations; and to Dr. G. N. Hoffer for permission to use the stalk-test data given in Tables 6, 7, 8, and 9. The stalk tests were made by W. P. Allyn.

[3] Reference is made by number (italic) to Literature Cited, p. 118.

One of the most recent methods proposed is that of Gilbert and Hardin (*5*), who suggest the composition of the sap of plants as a guide to available nutrients in the soil. These investigators found that the quantities of nitrate nitrogen, phosphate phosphorus, and potassium in the expressed sap of several garden and field crops paralleled the fertilizer treatments fairly well. Tentative critical concentrations which should be maintained in the saps of certain plant parts have been suggested. Concentrations below these levels indicate deficiencies in the soil. The investigations of McCool and his associates (*2, 3, 9, 10*) at the Michigan station, which quickly followed that of Gilbert and Hardin, have shown that fertilizer applications markedly affect the chemical composition of the expressed sap of various crop plants. These workers, however, are noncommittal concerning the use of this relation as a guide to fertilizer practices.

The results presented by the above investigators on sap composition are so encouraging as a possible means of detecting nutrient deficiencies as to merit thorough trial and investigation. The fact that nearly half a million tons of fertilizer are applied annually by Virginia farmers makes it very important that the method be given a thorough trial under Virginia crop, soil, and climatic conditions. The present paper contains some of the preliminary results obtained at the Virginia Agricultural Experiment Station to determine whether the sap of corn plants might be used as a guide to the nutrient needs of this crop. The observations reported here extend through two growing seasons, namely, 1928 and 1929.

MATERIALS AND METHODS

The plants from which sap was extracted for this investigation were grown on the continuous corn plot and the rotation with fertilizer plots of the Virginia Agricultural Experiment Station, Blacksburg, Va. These plots were started in 1909 and have, therefore, passed through 21 cropping seasons. The continuous corn plot (plot 1) has been cropped to corn every year, while the rotation plots (plots 2 to 14, inclusive) carry in rotation the following crops: First year, corn; second year, wheat; third and fourth years, hay (mammoth clover, red top, and timothy). The rotation experiment extends over four series of plots, which are designated as series A, B, C, and D.

In 1914 the continuous corn plot was divided in half. From 1914 to the present time the north half has received no treatment, while the south half has received a specified fertilizer treatment. The rotation plots were not divided until 1917. The south halves of these plots continued to receive the specified fertilizer treatment, while the north halves, which previously had received the same treatment as the south halves, were left unfertilized. As originally started in 1909, all plots were 165 by 33 feet in dimension, or an eighth of an acre in area. The division into halves, therefore, reduced them to 82½ by 33 feet, a sixteenth of an acre. The plots within each series were separated by 6-foot grassed alleys and the four series of plots were separated by grassed roadways 9 feet wide.

The kinds and amounts of fertilizers applied are given in Figure 1. During the first nine years (1909–1917), with the exception of manure, the fertilizer applications were one-half of that given in Figure 1. All applications are annual except as otherwise noted. For the plots receiving commercial materials, potassium has been applied as muriate

$1A_1$	1909–1930, no treatment.	$1A_2$	1909–1914, same as $1A_1$. 1914–1930, 16 tons manure and 219 pounds rock phosphate.
$2D_1$	1909–1917, same as $2D_2$. 1917–1930, no treatment.	$2D_2$	1909–1930, 438 pounds superphosphate.
$3D_1$	1909–1917, same as $3D_2$. 1917–1930, no treatment.	$3D_2$	1909–1930, 308 pounds dried blood, 438 pounds superphosphate, and 200 pounds muriate of potash.
$4D_1$	1909–1930, no treatment. Check.	$4D_2$	1909–1917, no treatment. 1917–1930, 200 pounds sulphate of ammonia, and 219 pounds rock phosphate.
$5D_1$	1909–1917, same as $5D_2$. 1917–1930, no treatment.	$5D_2$	1909–1930, 438 pounds superphosphate, and 200 pounds muriate of potash.
$6D_1$	1909–1917, same as $6D_2$. 1917–1930, no treatment.	$6D_2$	1909–1930, 200 pounds muriate of potash.
$7D_1$	1909–1917, same as $7D_2$. 1917–1930, no treatment.	$7D_2$	1909–1930, 308 pounds dried blood, and 200 pounds muriate of potash.
$8D_1$	1909–1917, same as $8D_2$. 1917–1930, no treatment.	$8D_2$	1909–1930, 308 pounds dried blood, and 438 pounds superphosphate.
$9D_1$	1909–1917, same as $9D_2$. 1917–1930, no treatment.	$9D_2$	1909–1930, 308 pounds dried blood.
$10D_1$	1909–1917, same as $10D_2$. 1917–1930, no treatment.	$10D_2$	1909–1930, 16 tons manure once in 4 years before corn.
$11D_1$	1909–1930, no treatment. Check.	$11D_2$	1909–1917, no treatment. 1917–1930, 16 tons manure once in 4 years before corn, and 219 pounds rock phosphate.
$12D_1$	1909–1917, same as $12D_2$. 1917–1930, no treatment.	$12D_2$	1909–1930, 4 tons manure annually.
$13D_1$	1909–1917, same as $13D_2$. 1917–1930, no treatment.	$13D_2$	1909–1930, 16 tons manure once in 4 years before corn, and 438 pounds superphosphate annually.
$14D_1$	1909–1917, same as $14D_2$. 1917–1930, no treatment.	$14D_2$	1909–1930, 219 pounds rock phosphate.

FIGURE 1.—Diagram of field plots used in the investigation, showing fertilizer treatments applied per acre

of potash, phosphorus as raw rock phosphate and superphosphate, and nitrogen as dried blood and sulphate of ammonia. Ground limestone has been applied to all plots every fourth year at the rate of 2 tons per acre. The last application was made in 1926.

The soil on which the plots used in this investigation are located is a Hagerstown silt loam of moderate fertility.

An open-pollinated strain of the Silver King variety of corn was used. The plants were spaced 20 inches apart in the row and the rows were 40 inches apart.

The work reported in this paper deals only with the studies made in 1928 and 1929. In 1928 saps were extracted on two dates, August 29 and September 28. In 1929 only one extraction was made, on August 30.

In gathering plants from which sap was to be obtained, care was always taken to select only plants which were typical of the corn-plant populations of the plots. Enough plants were taken to provide an abundance of sap for the various tests to be made. This usually required 10 or 12 plants from the more fertile plots and 15 to 20 from plots of low productiveness. After the plants were pulled they were taken to the laboratory for physical measurements and for sap extraction.

The sap samples were obtained from 15-inch sections of the stalks immediately above the surface of the soil. The stalks were cut at the ground line and at 15 inches above the ground line, the leaves and leaf sheaths were taken off, and any adhering soil particles were removed by washing. The wash water was then removed with a clean towel. The 15-inch sections were cut into small pieces approximately an inch in length, placed in a small Carver laboratory hydraulic press, and subjected to a pressure of 6,500 pounds per square inch. The saps obtained were measured, clarified with carbon black, and then stored in a refrigerator at $-7°$ C., where they remained in a frozen condition until removed for analysis. A small amount of toluene was added to each sample to prevent fermentation.

Nitrate nitrogen concentrations were determined by the phenol-disulphonic acid method. In clarifying and decolorizing the saps previous to determining the nitrate content, an attempt was made at first to use the procedure reported by Gilbert (*4*). Difficulty was experienced, however, in obtaining clear extracts and also in developing the colored solutions free from brownish tints. A method developed by Hill (*6*) was then tried and adopted. Total phosphorus was determined by the magnesium nitrate method as given by the Association of Official Agricultural Chemists (*1*, *p. 32*). Potassium was determined by the Lindo-Gladding method as slightly modified by R. P. Bartholomew, of the Arkansas experiment station.[4] No moisture determinations were made.

QUANTITY OF SAP AS RELATED TO SOIL PRODUCTIVITY

During the process of sap extraction it was noted that the sap flowed much more freely from some stalk samples than from others. Casual observation indicated that the large yields of sap were coming from samples made up of large, vigorous stalks, and that the small yields of sap were coming from stalks which had made poor growth. The larger stalks, of course, were produced on the more fertile plots. The yields of sap, therefore, appeared to be related to diameter of stalk and also to soil productiveness. Although this relation was noted in 1928, no measurements were made of the yields of sap. In

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1929, however, quantitative measurements were recorded, and these are reproduced in Table 1.

TABLE 1.—*Sap, stalk, and grain yield data from corn plants grown in 1929 on plots receiving different fertilizer treatments*

Plot No.	Treatment *	Stalk sections in sample	Total yield of sap	Yield of sap per section	Average diameter of stalks	Average volume of stalks	Sap per cubic centimeter of stalk volume	Yield of grain per acre
		Number	*C. c.*	*C. c.*	*Mm.*	*C. c.*	*C. c.*	*Bushels*
$1A_1$	Check	20	400	20.0	16.1	77.5	0.257	15.18
$1A_2$	M, Pr	12	590	49.2	27.7	226.2	.217	30.44
$2C_1$	O	10	350	35.0	26.8	215.0	.163	52.62
$2C_2$	Ps	10	425	42.5	29.4	258.5	.164	54.23
$3C_1$	O	10	435	43.5	28.1	236.3	.184	52.47
$3C_2$	N, P, K	10	719	71.9	26.9	216.5	.332	62.24
$4C_1$	Check	12	510	42.5	25.3	191.5	.222	42.30
$4C_2$	N (ams), Pr	12	480	40.0	25.2	189.9	.210	43.48
$5C_1$	O	12	400	33.3	23.6	166.6	.199	46.84
$5C_2$	Ps, K	10	835	83.5	30.6	280.1	.298	63.70
$6C_1$	O	12	520	43.3	25.0	187.0	.232	45.96
$6C_2$	K	10	680	68.0	28.7	246.4	.276	58.60
$7C_1$	O	11	460	41.8	25.0	187.0	.224	44.55
$7C_2$	N, K	8	605	75.6	30.6	280.1	.270	58.22
$8C_1$	O	11	445	40.5	27.2	221.3	.183	47.62
$8C_2$	N, Ps	12	540	45.0	27.4	224.5	.200	25.61
$9C_1$	O	14	330	23.6	22.2	147.4	.160	26.16
$9C_2$	N	15	440	29.3	23.1	159.6	.184	15.90
$10C_1$	O	14	370	26.4	23.0	158.3	.167	29.69
$10C_2$	M	10	835	83.5	30.1	271.1	.308	67.93
$11C_1$	Check	14	440	31.4	24.5	179.5	.175	23.37
$11C_2$	M, Pr	10	855	85.5	31.9	304.5	.281	66.91
$12C_1$	O	13	390	30.0	24.2	175.2	.171	31.10
$12C_2$	M	10	805	80.5	30.1	271.1	.297	65.45
$13C_1$	O	13	370	28.5	22.2	147.4	.193	36.08
$13C_2$	M, Ps	10	925	92.5	32.0	306.4	.302	59.04
$14C_1$	O	13	455	35.0	21.8	142.2	.246	32.27
$14C_2$	Pr	15	520	34.7	24.1	173.8	.200	35.05

* O, no treatment since 1917; check, no treatment since 1909; N, nitrogen from dried blood; N (ams), nitrogen from ammonium sulphate; M, manure; Pr, rock phosphate; Ps, superphosphate; K, muriate of potash.

The data presented in Table 1 strongly confirm the observations made in 1928. The yields of sap from the various plots range from 20 c. c. per stalk section to 92.5 c. c. per section. The largest yields of sap were obtained from the plots that received either muriate of potash or farm manure alone or in combination with other materials. These plots also produced the largest yields of grain and stalks of large diameter. The smallest yields of sap were obtained from the plots that received no fertilizer treatment. These plots generally produced stalks of small diameter, and returned very low yields of grain. The relationship between these characters is maintained by the plots of medium fertility, which yielded fair, although not large, quantities of sap. It is apparent, therefore, that the quantity of sap which may be pressed from a cornstalk bears a close relation to the fertility of the soil producing the stalk. The strength of this relation in the data presented in Table 1 is shown by the correlation coefficient 0.831 ± 0.040 between the yields of grain and the yields of sap. The diameter of stalk and the yields of sap are correlated to the extent of 0.857 ± 0.033.

These relations bring up the question whether the larger stalks yielded more sap because of their larger stalk volume or because they contained more sap per unit of volume. To determine which influence was stronger, the cubic centimeters of sap per cubic centimeter of stalk volume were calculated and included in Table 1. In

general, the larger stalks yielded more sap per unit of stalk volume than the smaller stalks. This relation, however, is far from perfect, as shown by the correlation coefficient 0.536 ± 0.091 between diameter of stalk and cubic centimeters of sap per unit of stalk volume. Since this relation is not as strong as the one mentioned above between diameter of stalk and yield of sap, it is apparent that the larger stalks gave up more sap primarily because of a greater stalk volume and secondarily because they contained more sap for each unit of stalk volume.

COLOR OF SAP AS RELATED TO SOIL DEFICIENCIES

As the sap samples came from the press they contained small pieces of stalk tissue that had been forced through the openings in the steel pressure cylinder which contained the stalk-tissue sample. Furthermore, the sap samples were of a dull-green color. In some of the samples, however, the green seemed to be partially masked by a brown color. Before chemical analyses could be made of the saps it was, of course, necessary to remove the solid matter which they contained. For the colorimetric nitrate determinations it was n cessary to remove the colors also. In some of the preliminary attempts to clarify and decolorize the samples 100 c. c. of sap were shaken for 15 minutes with 1 gm. of carbon black and filtered. The filtrates obtained were clear, but not colorless. The green color had been removed, but most of the brown still remained. The amount of brown, however, varied enormously among the different samples.

During 1928 it was noted that the saps coming from the unfertilized halves of the plots were a deeper shade of brown than those coming from the treated halves, except where the fertilizer treatment proved to be ineffective in maintaining fertility. This seemed to indicate a relation between the color of sap and soil productiveness. However, no method was available at the time for recording the shades of color. In 1929 the sap samples showed about the same variations in brown color with respect to fertilizer treatments and soil productivity. These samples, together with those expressed on September 28, 1928, were then taken to a commercial artist, who reproduced on canvas the color of each sample of sap after preliminary clarification. The reproductions are shown in Plate 1.

Considering Plate 1 as a whole, two things are immediately noticeable: (1) But one color is represented, namely, brown, and (2) the saps from plants grown on different plots show much variation in intensity of color, ranging from a total lack of brown to almost black. In general the saps of 1928 are darker than those of 1929. This difference may be due to the fact that the 1928 saps were a year old when taken to the artist, and therefore had probably undergone some oxidation even though they were kept in a frozen condition. The 1929 saps were only a week old when reproduced by the artist.

In comparing the fertilized and unfertilized ends of the plots it will be found that the saps coming from the unfertilized ends are on the whole much darker in color. This is true in both years. The saps coming from the unfertilized ends of some of the plots are lighter in color than those coming from the fertilized ends of some of the other plots, but almost without exception the untreated ends are darker than the corresponding treated ends. These observations suggest a fairly strong relation between the intensity of brown color

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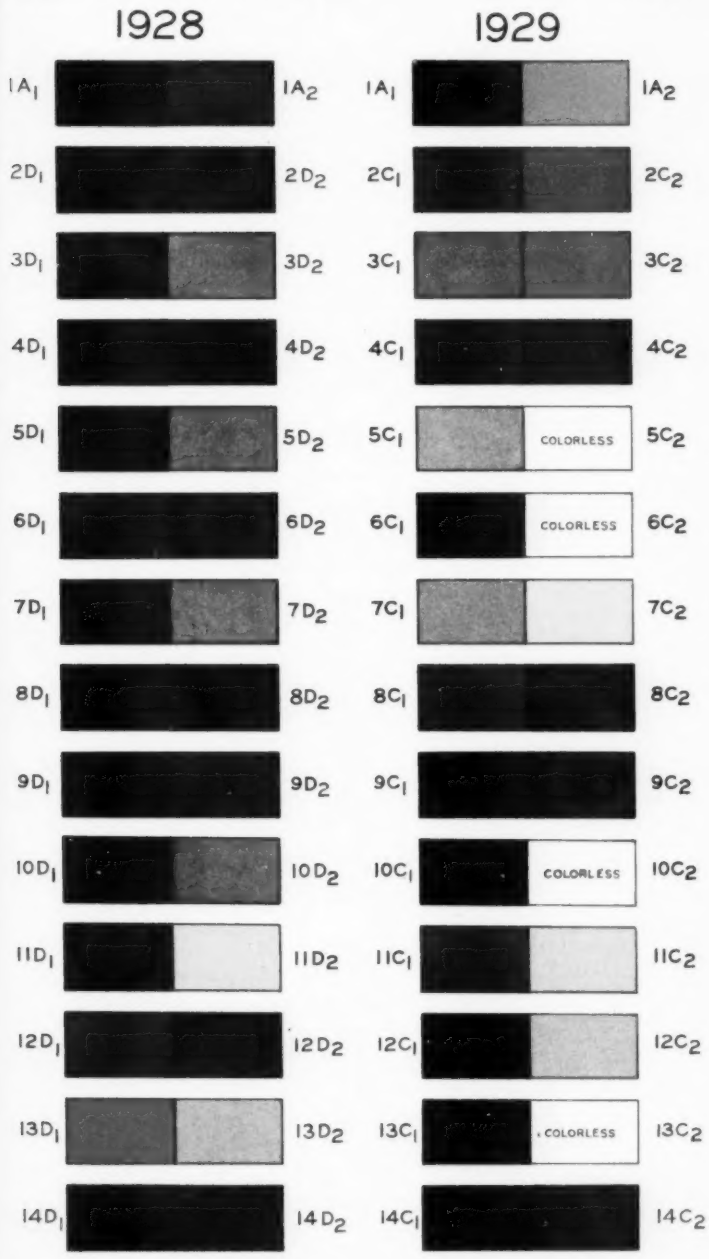

Color of corn-plant saps after preliminary clarification
The plants were grown on plots receiving different fertilizer treatments
(See Figure 1 for explanation of treatments given)

A. Hoen & Co. Baltimore, Md.

in the saps and fertilizer practices. A close examination of the fertilized halves of the plots, however, shows a number of exceptions. Thus, the saps from plots 2, 4, 8, 9, and 14 are conspicuously dark in color in the series of fertilized ends. These plots received fertilizer treatments which were relatively ineffective as judged by crop yields. The remaining plots, which yielded light-colored saps, received relatively effective fertilizer treatments, with the exception of plot 6 and possibly plot 7. Hence there appears to be a negative relation between soil productiveness and the intensity of color in the expressed sap. The more productive plots yielded colorless or light-brown saps, while the less productive plots are characterized by dark-brown saps. By assigning numerical values ranging from 0 to 7, inclusive, to the different intensities of color, the strength of this relation was found to be -0.523 ± 0.093 for 1928 and -0.804 ± 0.045 for 1929.

The sap colors also show a strong correlation with potassium fertilization. The treated ends of plots 2, 4, 8, 9, and 14 received no potassium, and the saps coming from these plots are dark in color. The fertilized ends of the remaining plots received potassium either as muriate of potash or in farm manure. The saps coming from these plots are either colorless or a light shade of brown. The only exceptions to this relation are the fertilized ends of plots 1, 6, and 12 in 1928. The saps from the unfertilized ends of all plots are dark in color and of course have received no potassium. Plate 1, therefore, shows that the color of the expressed saps of corn plants after clarification bears a close relation to the application and nonapplication of potassic fertilizers. Further work will be necessary to determine whether this relation can be used as a guide to the amount of available potassium in field soils and to the potassic needs of the corn plant.

QUANTITATIVE CHEMICAL COMPOSITION OF SAP AS RELATED TO FERTILIZER TREATMENT

The main objective of the work reported in this paper was to determine whether the composition of the sap is influenced by the application of fertilizers, and if so, whether the influence is sufficiently strong to permit the composition of the sap to be used as a guide to fertilizer practices. Hence, it was necessary to determine whether nitrogenous, phosphatic, and potassic fertilizers increase the nitrogen, phosphorus, and potassium content of the sap, respectively.

NITRATE NITROGEN

Although it is recognized that many different forms of nitrogen exist in plants, it was decided to limit the preliminary work reported here to the nitrate form.

As was pointed out under methods of chemical analysis, considerable difficulty was experienced in decolorizing the sap samples for the colorimetric phenoldisulphonic acid determination of nitrates. The improved procedure finally adopted was not available in 1928, and hence the nitrate results obtained during the first year are not considered to be worthy of presentation. The 1929 results are given in Table 2.

It is clear from Table 2 that the nitrate nitrogen content of the sap samples from the various plots differed enormously. The continuous

corn plot contained but 20 parts of nitrate nitrogen to a million of sap, while the fertilized half of plot 13 yielded a sap containing 1,110 parts per million. Concentrations ranging from one extreme to the other were observed.

TABLE 2.—*Nitrate nitrogen, total phosphorus, and total potassium contents of sap extracted from corn plants grown on plots receiving different fertilizer treatments in 1928 and 1929, and yields of grain per acre*

Plot No.	Treatment [a]	Nitrate nitrogen	P_2O_5 per cubic centimeter of sap extracted on—			K_2O per cubic centimeter of sap extracted on—			Yield of grain per acre	
			Aug. 29, 1928	Sept. 28, 1928	Aug. 30, 1929	Aug. 29, 1928	Sept. 28, 1928	Aug. 30, 1929	1928	1929
		P. p. m.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Bushels*	*Bushels*
$1A_1$	Check	20	0.159	0.175	0.197	0.74	1.26	0.47	6.85	15.18
$1A_2$	M, Pr	24	.128	.222	.165	3.52	4.01	3.47	15.71	30.44
$2D_1$[b]	O	160	.191	.138	.195	.59	1.92	2.38	14.72	52.62
$2D_2$	Ps	149	.361	.522	.277	.58	1.20	1.20	20.71	54.23
$3D_1$	O	177	.115	.186	.081	.71	1.59	2.11	23.14	52.47
$3D_2$	N, Ps, K	200	.231	.718	.219	5.94	5.60	4.03	15.14	62.24
$4D_1$	Check	165	.111	.296	.053	.48	1.24	.97	21.00	42.30
$4D_2$	N(ams), Pr	220	.209	.351	.127	.36	1.18	.74	24.43	43.48
$5D_1$	O	476	.161	.141	.060	.76	1.69	1.32	25.43	46.84
$5D_2$	Ps, K	250	.249	.952	.165	6.00	6.26	7.17	22.29	63.70
$6D_1$	O	278	.138	.168	.045	2.19	2.56	1.57	20.71	45.96
$6D_2$	K	370	.150	.282	.043	5.39	4.24	5.52	34.43	58.60
$7D_1$	O	556	.076	.144	.028	.52	2.31	2.87	16.57	44.55
$7D_2$	N, K	714	.117	.292	.042	5.62	5.97	4.90	34.57	58.22
$8D_1$	O	228	.179	.272	.050	.08	1.09	1.14	10.57	47.62
$8D_2$	N, Ps	322	.361	.509	.158	.44	.79	.83	13.57	25.61
$9D_1$	O	148	.126	.278	.105	.61	.79	.56	8.00	26.16
$9D_2$	N	220	.217	.272	.045	.39	.68	.54	7.58	15.90
$10D_1$	O	250	.136	.165	.076	.53	.85	.54	18.57	29.69
$10D_2$	M	1,000	.248	.440	.110	6.24	7.17	4.50	39.72	67.93
$11D_1$	Check	312	.114	.275	.098	.45	.85	.35	10.86	23.37
$11D_2$	M, Pr	714	.244	.347	.151	6.35	7.87	5.72	45.14	66.91
$12D_1$	O	303	.107	.254	.085	.90	1.96	.50	26.43	31.10
$12D_2$	M	768	.258	.467	.138	6.40	6.98	5.58	51.43	65.45
$13D_1$	O	312	.080	.099	.019	.72	.60	.56	22.15	36.08
$13D_2$	M, Ps	1,110		.471	.239		7.15	5.04	24.28	59.04
$14D_1$	O	137	.083	.113	.073	.18	1.55	1.10	17.43	32.27
$14D_2$	Pr	626	.110	.361	.036	.51	.91	.76	24.14	35.05

[a] O, no treatment since 1917; check, no treatment since 1909; N, nitrogen from dried blood; N(ams), nitrogen from ammonium sulphate; M, manure; Pr, rock phosphate; Ps, superphosphate; and K, muriate of potash.

[b] Plots 2–14, inclusive, on range D in 1928 and on range C in 1929.

With respect to nitrogen fertilization the nitrate concentrations show only a fair degree of correlation. Although the plots which received nitrogenous fertilizers generally yielded saps of higher nitrate content, there are a number of exceptions among the no-nitrogen plots. The data show further that the saps from plants grown on the continuous corn plots contained less than 25 p. p. m. of nitrate nitrogen, while the saps from plants grown on the rotation plots contained more than 150 p. p. m. The yields of grain and the general appearance and condition of the crop indicate that the nitrate supply was adequate in all plots except the two continuously cropped to corn. Two other tests to be discussed later confirm this statement.

The relatively high nitrate level in the saps from plants grown on the rotation plots resulted from high nitrate production in the soil. The high nitrate production was due to the fact that the season of 1929 was favorable for nitrification and that a clover crop (two

years) had preceded corn in the rotation. Even in August, when the demands for nitrogen are high, a composite soil sample from all plots revealed the presence of 25 pounds of nitrogen per acre in the nitrate form (12.5 p. p. m.).

TOTAL PHOSPHORUS

The sap samples of all three sampling dates were analyzed for total phosphorus. The results obtained are presented in Table 2.

These data show a remarkable correlation between the phosphorus content of the saps and phosphatic fertilization. On the first sampling date, August 29, 1928, the plots which received no phosphatic fertilizers yielded saps containing less than 0.20 mgm. P_2O_5 per cubic centimeter of sap. With but two exceptions, all plots to which phosphorus was applied gave saps with a phosphorus content above 0.20 mgm. P_2O_5 per cubic centimeter. The samples collected on September 28, 1928, show an even better correlation between the phosphorus content of the sap samples and fertilizer treatment. Plots which received no phosphatic fertilizers showed less than 0.30 mgm. P_2O_5 per cubic centimeter of sap. The plots which received raw rock phosphate, a relatively unavailable source of phosphorus, yielded saps containing between 0.30 and 0.40 mgm. per cubic centimeter. All plots which received superphosphate, a relatively available source of phosphorus, gave up more than 0.40 mgm. of P_2O_5 for every cubic centimeter of sap. The results for 1929 harmonize well with those of 1928. With but two exceptions the results correlate very well with the fertilizer treatments also. The application of phosphorus in any form increased the phosphorus content of the saps from 50 to 300 per cent.

There is much less phosphorus in the 1929 sap samples than in either the August or September samples of 1928. This low phosphorus level in 1929 may be explained by a larger demand for phosphorus as a result of more favorable conditions for growth. The season of 1928 was unfavorable for the growth of corn, as is shown by an average grain yield for all plots of only 22 bushels per acre. The favorable season of 1929 returned 44 bushels per acre as an average of all plots, an increase of 100 per cent over 1928. The phosphorus content of the sap was 0.173 and 0.110 mgm. (P_2O_5) per cubic centimeter in August of 1928 and 1929, respectively, a reduction of 36 per cent in 1929. These data indicate that the larger production of grain and stover in 1929 drew more heavily on the sap phosphorus than in 1928, thereby causing the observed reduction. The fact that a 100 per cent increase in grain yields was accompanied by a decrease of only 36 per cent in sap phosphorus may indicate that the phosphorus contained in the sap is used more efficiently when reduced to lower levels.

Table 2 brings out the effect of age of the corn plant on the phosphorus content of its sap. The saps expressed on September 28, 1928, contained nearly twice as much phosphorus as those obtained from plants from the same plots a month earlier. Furthermore, the samples taken on August 30, 1929, showed much less phosphorus than the September samples of 1928. These results are of interest in relation to the condition of the plants on the various sampling

dates. On August 29, 1928, the ears were fully developed, but had undergone little, if any, maturation. A month later the ears and leaves were fully mature, but the stalks still contained considerable sap. The difference in phosphorus content on the different dates as pointed out above may be explained by the differential demand for phosphorus by the plant during the earing and maturation periods. During August, when the ears are developing rapidly, the demand for phosphorus is high, and the assimilative processes keep the phosphorus content of the sap at a relatively low level. As the plant approaches maturity the demands for phosphorus gradually lessen, assimilative processes decrease in activity, and with a subsequent lag in the cessation of absorption, phosphorus accumulates in the sap during the maturation period. This occurred regardless of the previous fertilizer treatment. It is possible that a part of the observed increase in concentration was due to (1) the loss of water during maturation, or (2) to the movement of sap phosphorus into the stem from other parts of the plant. The former suggestion is not believed to have had much influence, for there was more sap in the stalks in September than in August. No data were obtained nor observations made concerning the latter suggestion.

POTASSIUM

The potassium content of the sap samples from all three sampling dates show very good correlation with the application of fertilizers carrying potassium. As shown in Table 2, the untreated ends of all plots and the treated ends of plots which received no potassic fertilizers yield plants which are very low in the potassium content of the expressed saps. The plots which received potassium, however, either in farm manure or as muriate of potash, show a higher potassium content in the sap. A close examination of the data shows that the saps coming from plots which received potassium contained from two to twenty times as much potassium as did the saps coming from plants grown on the untreated half of the same plot.

Potassium, like phosphorus, shows a tendency to accumulate in the sap during the maturation period of the plant. Twenty-four of the twenty-seven half plots tested in 1928 show larger quantities of potassium in the sap on September 28 than on August 29. In general, the increases were larger on the untreated halves. The potassium increases are not as large, however, as those of phosphorus for the same period.

CRITICAL NUTRIENT CONCENTRATIONS IN THE SAP

Although it is not possible to establish definitely from the two years of preliminary work reported here what quantities of nitrogen, phosphorus, and potassium should be present in the sap of the lower portion of cornstalks to insure maximum yields, the data suggest in a tentative way certain critical concentrations. Nitrate nitrogen should be present to the extent of at least 200 p. p. m.; higher concentrations, however, are desirable. Soils are generally deficient in available phosphorus when the sap contains less than 0.10 mgm. total phosphorus (P_2O_5) per cubic centimeter. From 0.10 to 0.20

mgm. P_2O_5 per cubic centimeter of sap gave average yields, while more than 0.20 mgm. generally gave good yields. The supply of available potassium in the soil is definitely deficient if the total potassium content of the sap is less than 1 mgm. K_2O per cubic centimeter, and generally so when it is less than 1.5 mgm. When the sap contained from 1.5 to 2 mgm. K_2O per cubic centimeter, average yields were obtained, while more than 2 mgm. indicated an ample supply of available soil potassium.

These tentative concentrations will be either confirmed or modified by further work. The nutrient concentrations which will be chosen ultimately as standards in diagnosing soil needs will have to be selected from experiments covering a wide range of crop, soil, and climatic conditions. The sap of the plant occupies an intermediate position between the soil solution and the plant tissue. Its nutrient composition is determined, therefore, at one end of the system by the supply of available nutrients and the forces controlling their absorption, and at the other end by the demands of the metabolic processes for these nutrients. As conditions become favorable or unfavorable for the influx of nutrients into the sap of the plant or for their removal by metabolic processes, different nutrient concentrations appear in the sap and tend to reflect the net result of nutrient supply and demand. Ultimately it may be necessary to establish critical nutrient concentrations for different soil types, different types of seasons, different crops, etc.

RELATIVE ABSORPTION OF NUTRIENTS FROM SOILS RECEIVING DIFFERENT FERTILIZER TREATMENTS

Although the data presented above show a close relation between fertilizer practices and composition of sap, the question arises as to whether the concentration of materials in the sap indicates the proportionate removal of nutrients from the soils of the various plots. It has been shown by Table 1 that the plants grown on the various plots differ in the quantities of sap given up. Hence, it is possible that some of the sap samples are low in concentration because the nutrients are distributed throughout a large quantity of sap. Conversely, some samples may be high in concentration because the nutrients are confined to a small quantity of sap. The former condition would give an underestimation of the relative removal of soil constituents while the latter condition would give an overestimation. On the other hand, it is possible that saps of high concentration were given up in large quantities and saps of low concentration in small quantities. In this event, the differences between plots as shown by nutrient concentrations in the sap would be magnified when translated to quantities of nutrients absorbed by the crop.

To determine the extent to which sap concentration indicates the quantity of materials removed from the soil, the contents of the 1929 saps have been calculated to the acre basis, and the figures are given in Table 3. The data show that with the exception of nitrogen, the plots which yielded concentrated saps gave up large quantities of nutrients, and that the relatively dilute saps are accompanied by small amounts of nutrients per acre. This relation is very close

for phosphorus and potassium as shown by the correlation coefficients 0.874 ± 0.037 for phosphorus and 0.983 ± 0.004 for potassium. The data show further that the plots which received either manure or muriate of potash gave up larger quantities of nitrogen, phosphorus, and potassium than the individual sap concentrations indicate. Figure 2 shows that the concentrations underestimated the relative quantities of these elements removed from the soil from 12 to 34 per cent. The plots which received no manure or muriate of potash gave up smaller quantities of nitrogen, phosphorus, and potassium than the sap concentrations indicate. On these plots the sap con-

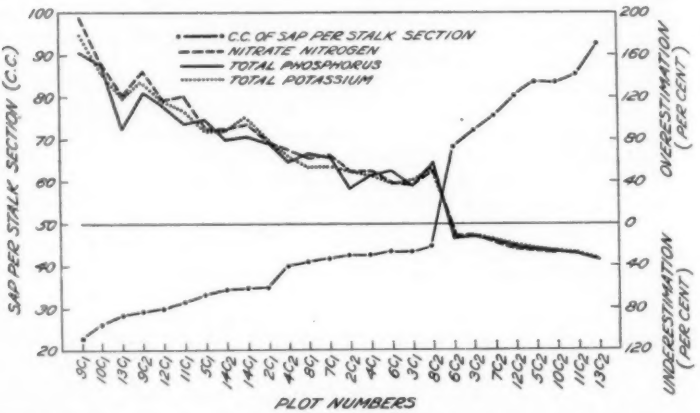

FIGURE 2.—Influence of quantity of sap yielded on the accuracy of nutrient concentrations in the sap as indicators of the relative removal of nutrients from soils receiving different fertilizer treatments.

centrations overestimated the relative nutrient removal from the various soils by 33 to 196 per cent.

Figure 2 shows also that the extent of overestimation and underestimation is inversely proportional to the yield of sap per stalk section. The stalk sections which yielded small quantities of sap overestimated the relative nutrient removal to the greatest extent. As the yields of sap increase, the overestimation decreases until a yield of approximately 60 c. c. of sap per stalk section is reached. Larger yields of sap resulted in an underestimation of the relative nutrient removal, and the larger the yield, the larger the underestimation. However, the underestimations do not increase as fast as the overestimations decrease for any given rise in sap yield.

TABLE 3.—*Nitrogen, phosphorus, and potassium content of sap extracted August 30, 1929, from 15-inch stalk sections of corn plants grown on plots receiving different fertilizer treatments*

Plot No.	Treatment [a]	Sap composition			Sap per 15-inch stalk section	Field stand (100 per cent equals 500 plants)	Sap contents per acre		
		Nitrate nitrogen	P_2O_5 per cubic centimeter of sap	K_2O per cubic centimeter of sap			Nitrate nitrogen as N.	P_2O_5	K_2O
		P.p.m.	*Mgm.*	*Mgm.*	*C. c.*	*Per cent*	*Pounds*	*Pounds*	*Pounds*
$1A_1$	Check	20	0.197	0.47	20.0				
$1A_2$	M, Pr	24	.165	3.47	49.2				
$2C_1$	O	160	.195	2.38	35.0	89.8	0.09	0.11	1.32
$2C_2$	Ps	149	.277	1.20	42.5	91.6	.10	.19	.82
$3C_1$	O	177	.081	2.11	43.5	92.8	.13	.06	1.50
$3C_2$	N, Ps, K	200	.219	4.03	71.9	90.6	.23	.25	4.63
$4C_1$	Check	165	.053	.97	42.5	91.6	.11	.04	.67
$4C_2$	N(ams), Pr	220	.127	.74	40.0	85.8	.13	.08	.45
$5C_1$	O	476	.060	1.32	33.3	90.8	.25	.03	.70
$5C_2$	Ps, K	250	.165	7.17	83.5	92.0	.34	.22	9.72
$6C_1$	O	278	.045	1.57	43.3	94.6	.20	.03	1.14
$6C_2$	K	370	.043	5.52	68.0	95.2	.42	.05	6.31
$7C_1$	O	556	.028	2.87	41.8	87.2	.36	.02	1.85
$7C_2$	N, K	714	.042	4.90	75.6	91.2	.87	.05	5.96
$8C_1$	O	228	.050	1.14	40.5	91.4	.15	.03	.74
$8C_2$	N, Ps	322	.158	.83	45.0	83.6	.21	.10	.55
$9C_1$	O	148	.105	.56	23.6	86.4	.05	.04	.20
$9C_2$	N	220	.045	.54	29.3	83.4	.09	.02	.23
$10C_1$	O	250	.076	.54	26.4	86.8	.10	.03	.22
$10C_2$	M	1,000	.110	4.50	83.5	92.4	1.36	.15	6.13
$11C_1$	Check	312	.098	.35	31.4	83.2	.14	.05	.17
$11C_2$	M, Pr	714	.151	5.72	85.5	93.0	1.00	.21	8.03
$12C_1$	O	303	.085	.50	30.0	85.0	.14	.04	.23
$12C_2$	M	768	.138	5.58	80.5	91.4	1.00	.18	7.25
$13C_1$	O	312	.019	.56	28.5	88.4	.14	.01	.25
$13C_2$	M, Ps	1,110	.239	5.04	92.5	91.4	1.66	.36	7.52
$14C_1$	O	137	.073	1.10	35.0	81.6	.07	.04	.54
$14C_2$	Pr	626	.036	.76	34.7	86.6	.33	.02	.40

[a] O, no treatment since 1917; check, no treatment since 1909; N, nitrogen from dried blood; N(ams), nitrogen from ammonium sulphate; M, manure; Pr, rock phosphate; Ps, superphosphate; K, muriate of potash.

RELATIVE EFFECTIVENESS OF FERTILIZERS USED AS SOURCES OF NITROGEN, PHOSPHORUS, AND POTASSIUM

Table 3 also shows the relative effectiveness of the various fertilizers used as sources of nitrogen, phosphorus, and potassium. The results are summarized for comparison in Table 4. The plots which received dried blood gave up more than twice as much nitrogen as those which received no nitrogenous fertilizers. Part of this good showing, however, was due to simultaneous additions of phosphatic and potassic fertilizers, as shown by the low yield of nitrogen by plot $9C_2$, which received dried blood alone. Manure proved to be the best source of nitrogen, yielding nearly four times as much nitrogen as dried blood and eight times as much as the no-nitrogen plots. With regard to sources of phosphorus, rock phosphate was ineffective, while manure alone and superphosphate alone yielded nearly five times as much as the no-phosphate plots. Manure and rock phosphate in combination yielded very little more phosphorus than manure alone, while manure and superphosphate yielded more phosphorus than any other material or combination of materials. Both muriate of potash and manure were very effective carriers of potassium, yielding about 10 times more potash than the plots which received no potassic materials.

TABLE 4.—*Summary comparisons of nitrogen, phosphorus, and potassium contained in sap extracted August 30, 1929, from 15-inch stalk sections of corn plants grown on plots receiving different fertilizer treatments*

Treatment	Sap contents per acre		
	Nitrate nitrogen as N.	P_2O_5	K_2O
	Pounds	*Pounds*	*Pounds*
Check [a]	0.15		
Dried blood	.35		
Ammonium sulphate	.13		
Manure	1.26		
Check [b]		0.04	
Rock phosphate		.05	
Superphosphate		.19	
Manure		.17	
Manure and rock phosphate		.21	
Manure and superphosphate		.36	
Check [c]			0.73
Muriate of potash			6.66
Manure			7.23

[a] Average of untreated ends of plots receiving nitrogen.
[b] Average of untreated ends of plots receiving phosphorus.
[c] Average of untreated ends of plots receiving potassium.

HYDROGEN-ION CONCENTRATION

In 1928 the samples collected on August 29 were tested for hydrogen-ion concentration. A small portion of the unclarified sap was used, the test being made immediately after extraction. A quinhydrone electrode was employed in making these determinations.

The results of these determinations, together with the yields of grain from the various plots, are given in Table 5. It may be noted that with the exception of plots 4, 9, and 12, the fertilized half of each plot shows a lower pH value than the corresponding unfertilized half. Plot 12 showed the same pH on both halves, while plot 4, which received rock phosphate and sulphate of ammonia, was definitely higher on the treated half, as was also plot 9, which received dried blood. It appeared, therefore, that there might be a relation between the pH value of the sap and soil productivity as measured by yields of grain. That such was not the case, however, is shown by the small and statistically insignificant coefficient of correlation -0.275 ± 0.118. There is a fairly consistent relation, however, between pH value and potassium fertilization. Nearly all of the plots which received potassium, either in farm manure or as muriate of potash, have given saps of high acidity. The nitrogenous and phosphatic fertilizers, when not applied with potassium, show relatively high pH values.

TABLE 5.—*Hydrogen-ion concentration of expressed saps and yields of grain from corn plants grown in 1928 on plots treated with different fertilizers*

Plot No.	Fertilizer treatment per acre	pH	Yield of grain per acre
			Bushels
$1A_1$	Continuous corn, no treatment	6.09	6.85
$1A_2$	Continuous corn, 219 pounds rock phosphate, 16 tons manure once in four years.	5.45	15.71
$2D_1$	No treatment	5.60	14.72
$2D_2$	438 pounds superphosphate	5.58	20.71
$3D_1$	No treatment	5.50	23.14
$3D_2$	308 pounds dried blood, 438 pounds superphosphate, 200 pounds muriate of potash.	5.36	25.14
$4D_1$	Check, no treatment	5.60	21.00
$4D_2$	200 pounds sulphate of ammonia, 219 pounds rock phosphate	5.79	24.43
$5D_1$	No treatment	5.55	25.43
$5D_2$	438 pounds superphosphate, 200 pounds muriate of potash	5.33	22.29
$6D_1$	No treatment	5.47	20.71
$6D_2$	200 pounds muriate of potash	5.31	34.43
$7D_1$	No treatment	5.70	16.57
$7D_2$	308 pounds dried blood, 200 pounds muriate of potash	5.38	34.57
$8D_1$	No treatment	5.89	10.57
$8D_2$	308 pounds dried blood, 438 pounds superphosphate	5.62	13.57
$9D_1$	No treatment	5.72	8.00
$9D_2$	308 pounds dried blood	5.94	7.58
$10D_1$	No treatment	5.72	18.57
$10D_2$	16 tons manure once in four years before corn	5.57	39.72
$11D_1$	Check, no treatment	5.67	10.86
$11D_2$	16 tons manure once in four years before corn, 219 pounds rock phosphate.	5.36	45.14
$12D_1$	No treatment	5.47	26.43
$12D_2$	4 tons manure annually	5.47	51.43
$13D_1$	No treatment	5.45	22.15
$13D_2$	16 tons manure once in four years before corn, 438 pounds superphosphate.	5.29	24.28
$14D_1$	No treatment	5.67	17.43
$14D_2$	438 pounds rock phosphate	5.50	24.14

AGREEMENT BETWEEN CERTAIN EXTERNAL PLANT CHARACTERISTICS ASSOCIATED WITH SOIL DEFICIENCIES, THE HOFFER STALK TEST, AND SAP COMPOSITION

For a long time agronomists have been searching for external plant symptoms indicative of soil deficiencies in the hope that laborious field and laboratory tests might be reduced to a minimum. It was recognized rather early that plants growing under nitrogen-deficient conditions are characterized by a light-green color. Attempts have been made also to assign poor grain development in the cereals to phosphorus deficiencies. It has been known for a long time that potassium is effective in preventing lodging in corn plants. Recently Hoffer (7) has stated that potassium-deficient soils cause the marginal tissues of the leaves of corn plants to die prematurely. He has shown also that this leaf "firing" is associated with iron accumulations in the nodal tissues of the plant. Since the results already presented with reference to the chemical composition of the sap as related to fertilizer treatments are so encouraging as a means of detecting nutrient deficiencies, it seemed worth while to determine whether certain external plant characteristics are associated with soil deficiencies as shown by the Hoffer stalk test and by the sap composition test.

COLOR OF PLANT

Notes were taken in both years on the color of plants and the general condition of the crop on the various plots. Table 6 shows that in 1928 the plots that received no nitrogen (except in crop residues) produced plants which were definitely of a light shade of green. The plants which received dried blood were nearly normal in color

while those which received either ammonium sulphate or manure were dark green. In 1929 the plants which received no nitrogenous fertilizers were generally normal green in color, while those which received nitrogen were dark green. (Table 7.) The normal green color in the no-nitrogen plots is explained by the very favorable conditions for nitrification in 1929 and the fact that clover preceded corn in the rotation. Even in August, when the demands for nitrogen are high, a composite soil sample from all plots showed 25 pounds of nitrate nitrogen per acre. It appears, therefore, that nitrates were sufficiently abundant in 1929 to enable the plants to make a normal growth and develop a normal color, even on the plots which received no nitrogenous materials other than the clover residues.

TABLE 6.—*General condition of crop, yield, color of corn plants, and Hoffer stalk tests as indicators of nitrogen needs in 1928 of corn grown on plots receiving different fertilizer treatments*

Plot No.	Treatment [a]	General condition of crop	Color of plants	Results of Hoffer NO_3 test [b]	Yield of grain per acre
					Bushels
$1A_1$	Check	Very poor		B^-, C^+, B^+, A^-, B^+	6.85
$1A_2$	M, Pr	Poor		C, A^-, A^-, B^-, C	15.71
$2D_2$	Ps	do	Light green	A, A, C, A^-, B	39.71
$3D_2$	N, Ps, K	Average	Light green to normal	B, B, C, A, C^+	25.14
$4D_2$	N(ams), Pr	Poor	Dark green	C, B^+, C, A, A^-	24.43
$5D_2$	Ps, K	Average	Light green	C, C, C, C, C	22.29
$6D_2$	K	Fair	do	C, C, C, B^+, A^+	34.43
$7D_2$	N, K	do	Light green to normal	A^-, A^-, B, C, A^-	34.57
$8D_2$	N, Ps	Poor	do	A, A^-, B^+, A^-, A^-	13.57
$9D_2$	N	do	do	A, A, A, A, B	7.58
$10D_2$	M	Good	Dark green	A, A, A^+, A, A^+	39.72
$11D_2$	M, Pr	do	do	B, B^-, B^-, A, A^+	45.14
$12D_2$	M	do	do	A^-, C, A^+, A, A^+	51.43
$13D_2$	M, Ps	do	do	B^+, A^-, B, B^+, B^+	24.28
$14D_2$	Pr	Poor	Light green to normal	B, B^+, A^-, A^-, B^-	24.14

[a] Check, no treatment since 1909; N, nitrogen from dried blood; N (ams), nitrogen from ammonium sulphate; M, manure; Pr, rock phosphate; Ps, superphosphate; K, muriate of potash.
[b] A, nitrates in abundance; B, nitrates present, but not in abundance; C, nitrates absent.

TABLE 7.—*General condition of crop, yield, color of corn plants, Hoffer stalk test, and nitrate nitrogen content of sap as indicators of nitrogen needs in 1929 of corn grown on plots receiving different fertilizer treatments*

Plot No.	Treatment [a]	General condition of crop	Color of plants	Results of Hoffer NO_3 test [b]	Nitrate nitrogen in sap	Yield of grain per acre
					P. p. m.	*Bushels*
$1A_1$	Check	Very poor	Light green	C, C, C, C	20	15.18
$1A_2$	M, P	Fair	do	C, C, C, C	24	30.44
$2C_2$	Ps	Average	do	C^+, B^-, C, C^+	149	54.23
$3C_2$	N, Ps, K	Good	Dark green	A, A, A, A	200	62.24
$4C_1$	Check	Fair	Normal green	A^-, A, A, A	165	42.30
$4C_2$	N(ams), Pr	do	do	A, A, A^-, A^-	220	43.48
$5C_2$	Ps, K	Good	do	C, A, A^-, A	250	63.70
$6C_2$	K	do	do	A, A, A, A, A	370	58.60
$7C_2$	N, K	do	do	A^+, A^+, A^+, A^+	714	58.22
$8C_2$	N, Ps	Poor	do	A, A, A, A	322	25.61
$9C_2$	N	do	Rusty green	A^-, A, A, A	148	15.90
$10C_2$	M	Very good	Dark green	A^+, A^+, A^+, A^+	1,000	67.93
$11C_2$	M, Pr	do	do	A^+, A^+, A^+, A^+	714	66.91
$11C_1$	Check	Poor	Rusty green	A, B, B^-, B	312	23.37
$12C_2$	M	Very good	Dark green	A^+, A^+, A^+, A^+	768	65.45
$13C_2$	M, Ps	do	do	A^+, A^+, A^+, A^+	1,110	59.04
$14C_2$	Pr	Poor	Rusty green	A^-, A^-, A, A	626	35.05

[a] Check, no treatment since 1909; N, nitrogen from dried blood; N(ams), nitrogen from ammonium sulphate; M, manure; Pr, rock phosphate; Ps, superphosphate; K, muriate of potash.
[b] A, nitrates in abundance; B, nitrates present, but not in abundance; C, nitrates absent.

On some of the plots the plants appeared to be of a "rusty" green color. This was caused by the large amounts of leaf firing occurring on these plots due to potassium starvation.

Table 6 shows that in 1928 there was fairly good agreement between plant color and the Hoffer stalk test as indicators of nitrogen needs. The light-green plants were in need of nitrogen as shown by the C nitrate readings. The plants of normal color tested A− to B, while the dark-green plants showed an abundance of nitrates by A+ to B+ readings.

In 1929 there was very good agreement between plant color, the results of the Hoffer stalk tests, and the nitrate-nitrogen concentration of the expressed sap. (Table 7.) Light-green plants were found only on plots $1A_1$, $1A_2$, and $2C_2$. The plants on plots $1A_1$ and $1A_2$ showed no nitrates by the stalk test (tested C), and the sap test showed less than 30 p. p. m. of nitrate nitrogen. The plants on plot $2C_2$ showed a trace of nitrates, and the sap showed 149 p. p. m. of nitrate nitrogen. Owing to the favorable season for nitrification, most of the plots contained sufficient nitrates for the production of normal color. These plots showed also a high nitrate content by both the stalk test and the sap test.

MARGINAL "FIRING" OF LEAF TISSUES

As stated previously, Hoffer has pointed out that dead tissues around the margins and between the veins of the leaves are associated with potassium deficiencies. He has shown also that iron accumulations in the nodal tissues of the plant are associated with a lack of available potassium. It seemed desirable, therefore, to make observations of the leaves on the various plots and to determine the relation of the amount of firing to the amount of potassium carried in the sap and to the degree of iron accumulations in the nodal tissues.

TABLE 8.—*Comparison of Hoffer stalk test and potassium content of sap of corn plants grown on plots differently fertilized, as indicators of potassium needs in 1928*

Plot No.	Treatment [a]	General condition of crop	Color of nodes [b]	Results of Hoffer Fe test [c]	K_2O per cubic centimeter of sap		Yield of grain per acre
					Aug. 29	Sept. 28	
					Mgm.	*Mgm.*	*Bushels*
$1A_1$	Check	Very poor	D, D, D, D, B	D^+, C^+, D^-, D^-, C	0.74	1.26	6.85
$1A_2$	M, Pr	Poor	Y, Y, Y, Y, B	C^-, C^-, C, C, C	3.52	4.01	15.71
$2D_2$	Ps	do	B, D, D, Y, B	C^+, D^-, D^-, C, C	.58	1.20	20.71
$3D_2$	N, Ps, K	Average	Y, Y, Y, Y, Y	C^-, C^-, C^-, C^-, C	5.94	5.60	25.14
$4D_2$	N(ams), Pr	Poor	D, B, B, D, B	D, D, C, C^+, C	.36	1.18	24.43
$5D_2$	Ps, K	Average	Y, Y, Y, Y, Y	C^-, C, C^-, C^-, C^-	6.00	6.26	22.29
$6D_2$	K	Fair	Y, Y, Y, Y, B	C^-, C, C^-, C, C	5.39	4.24	34.43
$7D_2$	N, K	do	Y, Y, B, Y, B	C^-, C^-, C, C^-, C	5.62	5.97	34.57
$8D_2$	N, Ps	Poor	D, B, B, D, D	D^+, D^-, D^-, D^-, D^-	.44	.79	13.57
$9D_2$	N	do	B, B, B, D, D	D, C, C, C^+, D	.39	.68	7.58
$10D_2$	M	Good	Y, B, Y, Y, Y	C^-, C^-, C^-, C^-, C	6.24	7.17	39.72
$11D_2$	M, Pr	do	Y, Y, Y, B, Y	C^-, C^-, C^-, C^-, C^-	6.35	7.87	45.14
$12D_2$	M	do	Y, Y, Y, Y, B	C^-, C^-, C^-, C^-, C^-	6.40	6.98	51.43
$13D_2$	M, Ps	do	Y, Y, Y, Y, Y	C^-, C^-, C^-, C^-, C^-		7.15	24.28
$14D_2$	Pr	Poor	D, D, D, D, B	E, D, D^-, D, C	.51	.91	24.14

[a] Check, no treatment since 1909; N, nitrogen from dried blood; N(ams), nitrogen from ammonium sulphate; M, manure; pr, rock phosphate; Ps, superphosphate; K, muriate of potash.

[b] Y, yellowish (normal); D, darkened; B, brown.

[c] C, slight accumulation (normal); D, medium accumulation; E, heavy accumulation.

The observations made in 1928 and 1929 are recorded in Tables 8 and 9, respectively. In both years the plots which received potash produced plants whose nodal tissues were free from discolorations, the iron accumulations were negligible, and the potash content of the expressed saps was high. Plants grown on plots which received no potash had badly discolored nodal tissues, showed heavy iron accumulations, and the expressed saps were very low in potash. Table 9 shows, in addition, that in 1929 the leaf firing followed closely the lack of potassium fertilization. (Fig. 3.) The plants which received

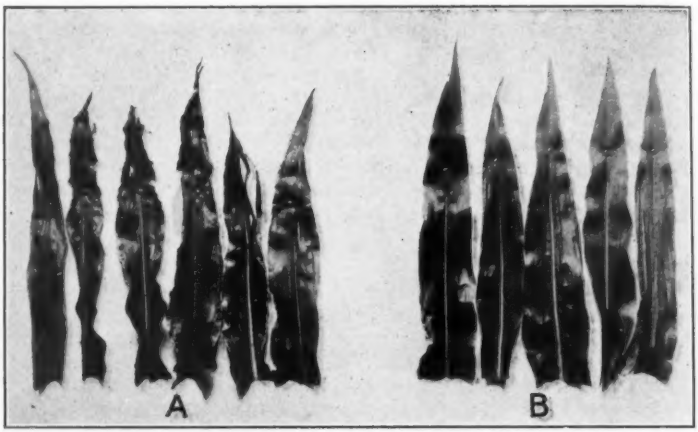

FIGURE 3.—Effect of potassium deficiency on the character of the leaf of the corn plant. Note that leaves in A, from plants which received nitrogen and phosphorus only, show dead tissues around their margins, indicating a lack of sufficient available soil potassium. Leaves in B, from plants which received a complete fertilizer in the form of farm manure, do not present this characteristic

no potash were badly fired, and the stalk and sap tests show these plots to be deficient in potassium. Plots which received applications of either manure or muriate of potash showed no firing, and the stalk and sap tests indicate that potassium was present in abundance. Hence, leaf firing, discoloration of the nodal tissues, iron accumulations in the nodal tissues, and small quantities of potassium in the sap all stand in agreement in indicating potassium deficiency. On the other hand, a lack of leaf firing indicates that the nodal tissues are free from discolorations and iron accumulations, that there are large quantities of potassium in the sap, and that potassium is not deficient in the soil.

TABLE 9.—*General crop condition, yield, marginal leaf firing, Hoffer stalk test, and potassium content of sap of corn plants grown on plots differently fertilized, as indicators of potassium needs in 1929*

Plot No.	Treatment [a]	General condition of crop	Leaf firing	Color of nodes [b]	Results of Hoffer Fe test [c]	K_2O per cubic centimeter of sap Aug. 30	Yield of grain per acre
						Mgm.	*Bushels*
$1A_1$	Check	Very poor	Bad	B, B, B, D	D^-, D^-, D, D	0.47	15.18
$1A_2$	M, P	Fair		Y, Y, Y, Y	C^+, C, C, C^+	3.47	30.44
$2C_2$	Ps	Average	Some	B, Y, D, B	D^-, D^-, E, C^+	1.20	54.23
$3C_2$	N, Ps, K	Good	None	Y, B, Y, Y	C^+, D^-, C, C	4.03	62.24
$4C_1$	Check	Fair	Some	D, B, B, B	D^+, C^+, C^+, D^-	.97	42.30
$4C_2$	N(ams), Pr	do	Bad	B, D, D, B	D^-, D^-, D^+, D	.74	43.48
$5C_2$	Ps, K	Good	None	Y, Y, Y, Y	C^-, C, C, D	7.17	63.70
$6C_2$	K	do	do	B, Y, Y, Y	C^+, C, C^-, C^-	5.52	58.60
$7C_2$	N, K	do	do	B, Y, Y, Y	C^+, C, C^-, C	4.90	58.22
$8C_2$	N, Ps	Poor	Bad	D, D, D, D	D^-, D, D, E	.83	25.61
$9C_2$	N	do	do	D, B, D, D	D, D^-, D^+, D^+	.54	15.90
$10C_2$	M	Very good	None	Y, Y, D, Y	C, C, D^-, C	4.50	67.93
$11C_2$	M, Pr	do	do	Y, Y, Y, Y	C^-, C, C, C^+	5.72	66.91
$11C_1$	Check	Poor	Bad	D, D, D, D	D^+, D, D, D	.35	23.37
$12C_2$	M	Very good	None	Y, Y, Y, Y	C, C, C^+, C	5.58	63.45
$13C_2$	M, Ps	do	do	Y, B, Y, Y	C^-, D^-, C^-, C	5.04	59.04
$14C_2$	Pr	Poor	Bad	D, B, B, D	C^+, C^+, D^-, E	.76	35.05

[a] Check, no treatment since 1909; N, nitrogen from dried blood; N(ams), nitrogen from ammonium sulphate; M, manure; Pr, rock phosphate; Ps, superphosphate; K, muriate of potash.
[b] Y, yellowish (normal); D, darkened; B, brown.
[c] C, slight accumulation (normal); D, medium accumulation; E, heavy accumulation.

TABLE 10.—*Percentage of leaning and broken stalks, and pulling resistance of corn plants grown in 1928 and 1929 on plots receiving different fertilizer treatments*

Plot No.	Treatment [a]	Leaning and broken stalks		Pulling resistance	
		1928	1929	1928	1929
		Per cent	*Per cent*	*Pounds*	*Pounds*
$1A_1$	Check	64.2	----------	60±4.0	----------
$1A_2$	M, Pr	64.8	----------	148±6.1	----------
$2D_1$ [b]	O	73.2	5.1	----------	----------
$2D_2$	Ps	62.6	3.4	134±5.5	168±9.4
$3D_1$	O	57.6	4.2	----------	----------
$3D_2$	N, Ps, K	38.8	1.8	198±6.6	270±16.7
$4D_1$	Check	41.5	11.8	118±4.6	128± 5.8
$4D_2$	N(ams), Pr	54.1	18.9	122±5.3	148± 7.2
$5D_1$	O	39.8	10.3	----------	----------
$5D_2$	Ps, K	31.3	1.6	222±5.8	206± 8.2
$6D_1$	O	76.3	5.0	----------	----------
$6D_2$	K	59.4	1.5	152±6.3	214±14.6
$7D_1$	O	62.3	2.6	----------	----------
$7D_2$	N, K	57.7	2.3	170±8.1	228±10.5
$8D_1$	O	64.9	11.3	----------	----------
$8D_2$	N, Ps	61.5	30.3	96±6.0	102± 8.4
$9D_1$	O	59.5	21.0	----------	----------
$9D_2$	N	67.9	33.7	90±6.2	134± 9.7
$10D_1$	O	40.8	29.0	----------	----------
$10D_2$	M	18.1	.9	260±5.4	220±11.3
$11D_1$	Check	50.0	21.9	96±5.1	120± 9.4
$11D_2$	M, Pr	22.6	.7	208±6.3	214±12.3
$12D_1$	O	34.7	25.7	----------	----------
$12D_2$	M	19.9	1.6	268±7.1	272±14.8
$13D_1$	O	47.9	28.0	----------	----------
$13D_2$	M, Ps	19.4	2.9	270±7.2	206±12.0
$14D_1$	O	57.4	18.7	----------	----------
$14D_2$	Pr	59.5	29.0	124±8.2	162±13.6

[a] O, no treatment since 1917; Check, no treatment since 1909; N, nitrogen from dried blood; N(ams), nitrogen from ammonium sulphate; M, manure; Pr, rock phosphate; Ps, superphosphate; K, muriate of potash.
[b] Plots 2 to 14, inclusive, on series D in 1928 and on series C in 1929.

LEANING AND BROKEN STALKS

It is generally believed that potassium is essential to the synthesis of carbohydrates in plants, and that it aids in the translocation of these substances to the various plant parts. Carbohydrates are known to be a major constituent of all of the structural tissues of plants. It is apparent then that potassium plays an important rôle in the proper development of the tissues that enable the corn plant to stand erect in the field; namely, the stalk and root tissues. If potassium actually performs these functions the plots used in the investigations reported here should show considerable difference in percentage of leaning and broken stalks, for some of the plots are now known to be deficient in potassium, while others are well supplied with this element. Furthermore, if lodging is due to potassium deficiency, this characteristic might be used as supplementary evidence in connection with other tests.

To determine the effect of potassium on the amount of lodging, field counts were made on all plots in 1928 and 1929. Plants were considered as leaning when they deviated more than 30° from the vertical position. The results, given in Table 10, show two outstanding relations. First, that corn lodged badly in 1928, but comparatively little in 1929. The large amount of lodging in 1928 was caused by somewhat excessive precipitation during most of the spring and summer months. The wet weather promoted the development of shallow root systems, which were unable to anchor the plants sufficiently in their later stages of development. The second important relation shown by Table 10 is the reduction in percentage of leaning and broken stalks on the plots which received potassium, either as muriate of potash or in farm manure. (Fig. 4.) This is shown to a greater extent by the 1929 data than by the data of 1928. The lodging on the potash plots was reduced to less than 3 per cent in 1929, while that on the plots without potash varied up to more than 30 per cent. The high percentages (1929) shown by plots $4C_2$, $8C_2$, and $9C_2$, on which nitrogen was applied without potash, seem to indicate that nitrogen increases lodging in corn unless counterbalanced by potassium. Such is not the case, however, for the data in Table 2 show that the saps coming from plants grown on these plots were not high in nitrates and contained less than 1 mgm. K_2O per cubic centimeter. The limiting factor here appears to be insufficient available potassium rather than high nitrogen.

Phosphorus, if judged by plot $2C_2$ in 1929, has a tendency to decrease lodging. Table 2, however, shows that here again potassium may have been the cause of the observed result. The sap from plants grown on plot $2C_2$ contained 1.20 mgm. K_2O per cubic centimeter. While this quantity is not large, it appears to have been sufficient to prevent excessive lodging. That the decrease in lodging was not due to phosphorus is well shown by the phosphated plots $8D_2$ and $8C_2$, where the percentages of leaning and broken stalks were relatively high in both years. A thorough study of the data in Tables 2 and 10 leaves no doubt as to the favorable effects of potassium in reducing the number of leaning stalks in corn. The 1928 data show that this is not an infallible guide, however, and should not be relied on as an indicator of potassium needs in seasons which promote the development of shallow root systems. In normal seasons the amount of lodging may be used to confirm information given by other tests.

Tables 2 and 10 also indicate that the critical minimum concentration for potassium in 1929 so far as lodging is concerned was approximately 1 mgm. K_2O per cubic centimeter of sap. This is evidenced by the fact that all of the 13 plots which showed less than 6 per cent lodging yielded plants whose sap contained more than 1 mgm. K_2O per

FIGURE 4.—The effect of a lack of potash on the ability of corn plants to stand erect is shown in A; potassium deficiency prevents good root anchorage and a large percentage of the plants lodge. The effect of an ample supply of available soil potash is shown in B; note how erect the plants are standing

cubic centimeter. Of the 13 plots which showed more than 10 per cent lodging 10 yielded plant saps containing less than 1 mgm. K_2O per cubic centimeter. This indicates that in the season of 1929 1 mgm. K_2O per cubic centimeter of sap marks approximately the dividing line between sufficient and insufficient potassium, so far as lodging is concerned.

ROOT ANCHORAGE

Whether or not a corn plant lodges is determined largely by how well the root system is anchored. Anchorage of the system, in turn, is determined by the number and extent of roots. Since potassium plays such an important rôle in keeping lodging at a minimum, it follows that root anchorage should be greater on plots receiving potassium than on similar plots receiving none.

To test this anticipated relation, 20 stalks were pulled from each plot by means of a pulling machine similar to the one described by Holbert and Koehler (*8*). This machine operates on the principle of a lever of the second class and measures the number of pounds necessary to remove a corn stubble from the soil by vertical pull. The plants to be pulled were selected at random.

Table 10 shows that potassium exerted a strong influence on the degree of root anchorage, for a much greater pull was required to remove plants which received either farm manure or muriate of potash than plants which received no potassium. Many of the differences between the mean pulling resistances of plants receiving potash or manure and those receiving none are significant statistically as measured by the probable error of the difference method. There is some evidence that root anchorage was decreased by nitrogen and increased by phosphorus. The data are not consistent with reference to these relations, however, and what has been said above about the potassium content of the sap being the effective agent in explaining the percentages of leaning and broken stalks, is equally pertinent in explaining the pseudo effects of nitrogen and phosphorus on root anchorage. The data seem to warrant the conclusion that potassium is the most effective element in reducing lodging in corn, and that it accomplishes this by promoting root development and thereby giving the plant better anchorage and less opportunity to decline from the vertical position.

The pulling data show also that the plants were somewhat better anchored in 1929 than in 1928. This was to be expected in view of the shallow root development and excessive amounts of leaning and broken stalks which resulted from the peculiar weather conditions of the 1928 season mentioned above.

Hoffer's work (*7*) shows that the probable explanation of the observed effects of potassium on lodging and root anchorage lies in the ability of potassium to keep the vascular tissues of the plant operating normally. The large amount of lodging and poor root development on the potassium-deficient plots are due to the fact that the accumulation of iron compounds in the sap-conducting vessels at the nodes interferes with the translocation of manufactured foods from the leaves to the roots. The roots are therefore undernourished, develop poorly, and fail to anchor the plant adequately. The favorable effect of potassium comes in preventing the accumulation of iron compounds at the nodes and therefore permits a normal movement of foods to the roots, facilitates proper root development, and provides adequate anchorage of the plant. It should be kept in mind also that if potassium actually promotes carbohydrate synthesis, as is generally believed, its capacity to help provide an abundant supply of carbohydrates is equally as important as its ability to aid in transporting these materials to points of assimilation, for carbohydrates in plants must be manufactured before they can be transported.

SUMMARY AND CONCLUSIONS

The investigations reported here were undertaken to determine (1) whether certain characteristics of the expressed sap of corn plants are related to fertilizer practices, and if so, whether the relationships are sufficiently strong to be used as a basis for recommending the application of various fertilizers, and (2) to determine the extent of agreement between the results obtained and those obtained by other methods.

The saps were extracted with a small laboratory hydraulic press at a pressure of 6,500 pounds per square inch from 15-inch sections of stalks immediately above the ground.

The quantity of sap that can be extracted from the stalk tissues of corn plants is closely related to diameter of stalk and to soil productivity. Large stalks produced on fertile land yielded from 70 to 90 c. c. of sap per stalk section, while small stalks, from unproductive soil yielded only 20 to 30 c. c. per section. Large stalks contain more sap per unit of stalk volume than do small stalks.

There is a general relationship between the color of the expressed saps and the productiveness of the soil on which the plants are grown. Saps that are colorless after clarification, and those that are light brown in color indicate a fertile soil, while a dark-brown color in the sap is associated with unproductiveness.

The color of the expressed saps is closely correlated with potassium fertilization. Dark-brown saps were obtained from plants grown on soils that are deficient in available potassium, while colorless saps and those of the light shades of brown were obtained from plants grown on soils containing an abundance of available potassium.

The concentration of nitrate nitrogen showed only a fair degree of correlation with nitrogen fertilization. It was well correlated, however, with the soil supply of nitrate nitrogen.

The total phosphorus content of the sap is closely related to phosphatic fertilization. Applications of either superphosphate or farm manure increased the total phosphorus content from two to five times that contained in saps coming from plants grown on plots that received no phosphorus. Rock phosphate was less effective in increasing the phosphorus content than either superphosphate or manure.

Phosphorus concentrations were high when grain production was subnormal, and lower when grain production was normal to supernormal.

Because of the lessened demand for phosphorus after the ear has developed, phosphorus accumulates in the sap during September. The increase is approximately 100 per cent and occurs irrespective of phosphate fertilization.

The potassium content of the sap shows nearly perfect correlation with potassium fertilization. The saps coming from plants grown on plots which received either muriate of potash or manure contained from two to sixteen times more potassium than saps from plants grown on similar plots receiving no such treatment.

There is a slight tendency for potassium to accumulate in the sap during the month of September. The increases, however, are small compared to those of phosphorus for the same period.

The following approximate nutrient concentrations in the sap are suggested tentatively as standards in diagnosing soils: Very deficient, nitrate nitrogen less than 100 p. p. m., total phosphorus less than 0.10 mgm. P_2O_5 per cubic centimeter of sap, potassium less

than 1 mgm. K_2O per cubic centimeter; moderately deficient, nitrate nitrogen 200 p. p. m., total phosphorus 0.10 to 0.20 mgm. P_2O_5 per cubic centimeter, potassium 1.5 to 2.0 mgm. K_2O per cubic centimeter; ample, nitrate nitrogen above 300 p. p. m., total phosphorus above 0.20 mgm. P_2O_5 per cubic centimeter, and potassium more than 2 mgm. K_2O per cubic centimeter.

Although the concentration of nutrients in the sap is a good indicator of the relative quantities removed from the soil, there is a tendency to overestimate the removal on unproductive soils and to underestimate the removal on fertile soils.

The hydrogen-ion concentration of the expressed sap shows a fair correlation with potassium fertilization. In general, saps from plants grown on plots receiving potassium are below pH 5.45, while those from plots receiving no potassium are higher.

The hydrogen-ion concentration is not appreciably correlated with soil productivity.

The depth of green color shown by corn plants is a good indicator of nitrogen needs. The color of the plant, the results obtained by the Hoffer stalk test for nitrates, and the nitrate concentration in the sap all agree in indicating nitrogen needs in the tests conducted.

Dead tissues around the margins and between the veins of the leaves is an accurate indicator of potassium deficiency. This condition was well correlated with the Hoffer test for iron accumulations in the nodal tissues of the plant and small amounts of potassium in the sap. The absence of dead tissues in the leaves was accompanied by nodal tissues free from discolorations and iron accumulations and by large quantities of potassium in the sap.

Except in seasons which promote shallow root development, the amount and severity of lodging may be used as a guide to potassium needs.

Applications of potassium were accompanied by increased root anchorage. This was probably due to the prevention of iron accumulations at the nodes which allowed normal translocation of foods to the roots and therefore more extensive root development.

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SOME OF THE CHARACTERISTICS OF YEASTS FOUND IN FERMENTING HONEY [1]

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INTRODUCTION

Honey is produced for the market in two forms, so-called comb or section honey and extracted honey. In Wisconsin alone from 6 to 10 million pounds of extracted honey are produced each year. The common method of marketing honey is to extract it from the combs, strain, and put it in 5 and 10 pound pails for retail trade and 60-pound cans for wholesale trade. A part of the honey put in 5 and 10 pound pails is pasteurized by heating to 160° F., but by far the greater portion of the honey that goes into the wholesale market is not pasteurized. In from four to eight weeks after removal from the comb, the honey begins to crystallize and by the time it is delivered to the broker or wholesale dealer it is completely granulated. Under conditions as they existed a few years ago, honey found its way to the consumer within six to eight months after it was produced. Under present conditions of greatly increased production, it is often stored for many months, perhaps years, and, when stored under unfavorable conditions, may undergo decided changes in flavor and color which reduce it to an inferior market grade.

But still another problem of greater importance in the storage of honey is that of fermentation. The majority of beekeepers do not make any attempt to pasteurize honey and consequently fermented honey is of frequent occurrence. It was formerly supposed that when fermentation took place, the honey had not been properly prepared by the beekeeper or perhaps had been extracted from the comb before it was thoroughly ripened. However, many recent cases have been noted which show that fermentation is not a result of contamination due to unsanitary methods of handling. The organisms which cause the fermentation are found normally in association with bees and the bees are responsible for their spread in new honeys (*14*).[2] These organisms are not killed during the ripening of the honey, and therefore develop at certain temperatures when the physical conditions of the honey are changed through granulation.

REVIEW OF LITERATURE

Since honey fermentation and spoilage have not been of much economic importance until recent years, the literature on the subject is meager. A few papers have been published in scientific journals and a few references have been made to honey fermentation in popular journals on beekeeping.

[1] Received for publication Feb. 2, 1931; issued August, 1931.
[2] Reference is made by number (italic) to Literature Cited, p. 131.

At the request of the Beekeepers' Association of Ontario, Shutt (*12*) undertook to ascertain the difference in composition between honey taken from uncapped and capped combs. Honey from uncapped combs is known as immature or unripe. Honey from fully capped combs or ripe honey contains from 4 to 5 per cent less moisture than that from the partially or entirely uncapped combs. Shutt found that honey from the uncapped combs has decidedly poorer keeping qualities than that from fully capped combs.

Klöcker (*3*, *4*) isolated an organism which he named *Zygosaccharomyces priorianus* from the bodies and honey sacs of honeybees. The cells of this yeast were oval or elongated and were firmly bound together so that when sedimented the yeast formed a coherent mass. The spores were round or oval and generally numbered from two to four in a cell. This species fermented glucose, fructose, maltose, and sucrose.

Cook (*1*) states that unripe honey is an excellent medium for microorganisms, and that during fermentation there is an evolution of carbon dioxide. In a Chicago warehouse he found barrels of honey bursting as a result of the formation of this gas.

One of the early investigations of fermented honey was made by Nussbaumer (*9*), who worked with a sample of Canadian honey. From this he isolated two strains of *Zygosaccharomyces*, which he called strains A and B. The two strains fermented glucose, fructose, mannose, sucrose, and dextrin. Strain A fermented maltose while B did not, and B fermented galactose while A did not. In some of his later experiments Nussbaumer diluted 61 samples of honey and incubated these for several months. Strains of *Zygosaccharomyces* were obtained from all the samples that fermented—37 in number. He advised heating honey to 70° C. for 30 minutes to prevent fermentation.

Richter (*10*) observed the souring and fermentation of honey in the combs before it was removed from the hive and in cells sealed by the bees. The development of gas in the cells was so great that the cappings ruptured and the contents were forced out, flowing over the combs in foaming streams. From this fermenting comb honey he isolated *Zygosaccharomyces mellis acidi*. The fermentation products of this organism were alcohol and acid. This yeast fermented glucose, fructose, and sucrose strongly and galactose feebly.

According to Root (*11*), not one beekeeper in a hundred can tell by the taste or appearance of extracted honey whether or not it is proof against fermentation when placed in storage. It is impossible for all honey to reach the consumer within six to nine months from the time it is produced, and therefore the supreme test comes when honey is kept past the spring months. Root found that granulated honey kept in a warm room underwent fermentation, and as a result of gas production burst the cans. While the honey was not very sour to the taste, there had been enough change practically to ruin it for the market. The mere fact that honey is granulated is not by any means proof that it is entirely ripe or that it will keep.

Marvin (*7*, *8*) isolated five types of yeastlike organisms from samples of fermenting Wisconsin honey. All of these organisms were found capable of fermenting honey after it had granulated. When the honey was heated to 160° F. fermentation was prevented. Fabian and Quinet (*2*) isolated four species of Zygosaccharomyces and one strain of Torula from samples of fermenting honey. Three were iden-

tified as previously described species of Zygosaccharomyces, while two were regarded as new species. One of these was named *Zygosaccharomyces mellis* and the other *Torula mellis*.

Lockhead and Heron (*5*) isolated four types of yeasts from fermented honey and found that all belonged to the genus Zygosaccharomyces. Two types were identified with previously described yeasts, namely, *Z. barkeri* and *Z. mellis*. The other two were considered new and designated as *Z. nussbaumeri* and *Z. richgeri*.

EXPERIMENTAL WORK

FIELD OBSERVATIONS ON FERMENTED HONEY

Late in 1925 a study of the causes of fermentation in honey and methods of preventing this loss while honey is in storage was started. Since the beginning of this investigation 49 samples of fermented honey from the following regions have been received and studied: California 2, Colorado 1, Connecticut 1, Florida 1, Illinois 4, Kentucky 1, Maine 2, Ohio 1, Utah 3, Wisconsin 28, Hawaii 1, Norway 1, Porto Rico 1, and Canada 2. From these samples 50 cultures of yeast have been isolated and 5 of these have been studied in detail.

Tall glass containers were filled with the fermenting honey. All of the samples had a soft granulation. Soon they became plainly divided into a lower granulated layer and an upper fluid layer with the surface covered with foam. The odor resembled that of sweet wine or fermenting canned fruit, and on microscopic examination the honey showed many yeast cells. As fermentation progressed, the fluid layer increased in depth and the granulated layer decreased.

ISOLATION OF YEASTS FROM HONEY

At first some difficulty was encountered in getting a suitable medium for the growth of the honey yeasts, but after several preliminary experiments a medium was made up of the following composition:

Yeast water [1]	1,000 c. c.
Honey	200 c. c.
Agar	36 gm.

The agar was dissolved in the yeast water and the honey was added just before the medium was tubed or placed in 125 c. c. flasks. These flask cultures were for giant colonies and for pouring plates. The medium was then sterilized for 20 minutes at 15 pounds pressure. If sterilized for a longer time, the medium will become dark because of caramelization, the agar will be partly hydrolyzed by the acid of the honey, and the medium will not harden properly.

The reaction of this medium was about pH 4.5. As honey yeasts grow better on a slightly acid medium than on a neutralized one, the medium was never brought to neutrality.

Three different methods were used in isolating yeasts from a sample of honey. (1) A loopful of fermenting honey was thoroughly mixed with the melted honey agar and the mixture was poured into a sterile Petri dish and allowed to harden. (2) The medium was liquefied, poured into a sterile Petri dish, and allowed to harden.

[1] Starch-free yeast 100 gm. and 1,000 c.c. of water, steamed for two hours and sterilized. After standing for two weeks the clear supernatant liquid was used as a nutrient.

The hardened surface was then streaked with a needle which had been inserted previously in fermenting honey. (3) Ten or twelve loopfuls of fermented honey were put into a tube of sterile water, which was agitated to dissolve the honey, and centrifuged. The clear liquid on top was drawn off. A loopful of the heavier material on the bottom was used as an inoculum and the first procedure was followed.

The Petri dishes inoculated as described were incubated (1) at room temperature and at 28° C. under aerobic conditions, (2) in anaerobic jars at room temperature and at 28° C. and (3) under vacuum at room temperature and at 28°.

Within two or three days the plates incubated under aerobic conditions at 28° C. showed growth, while those incubated at room temperature required from five to six days for the colonies to appear. Microscopic examination revealed that the colonies were yeasts. No growth at all was noticed on the plates incubated in anaerobic or vacuum jars.

Plating operations were repeated many times and different samples of fermenting honey from various sources were used. Each time yeast colonies and molds were the only microorganisms that appeared on the plates. When some honeys were plated, so many molds appeared on the plate that the entire surface was covered before a yeast colony could be selected. Under these circumstances, a few loopfuls of fermenting honey were put in a sterile mixture of 50-50 honey and yeast water. When the mixture began to ferment, a loopful was plated out and usually no molds appeared.

Isolated colonies of yeasts were picked from the plates and transferred to slopes of the same medium. From these slopes, material was used for stock cultures and for further inoculation.

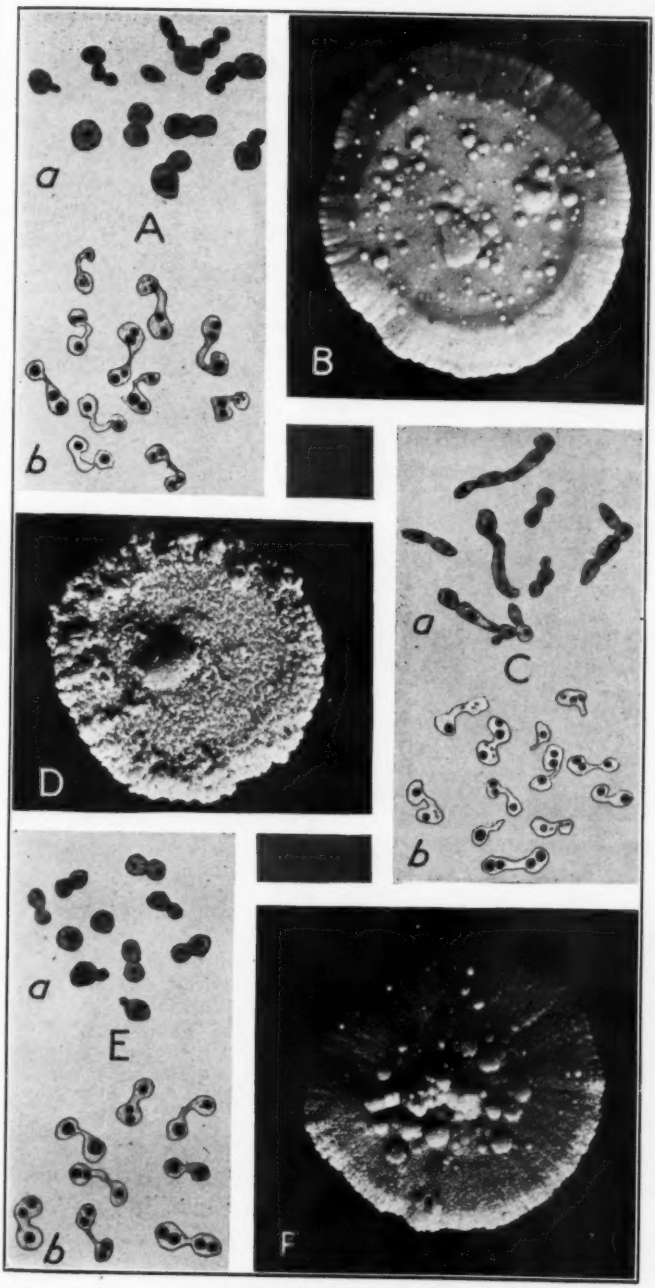
Giant colonies of each yeast were grown. Erlenmeyer flasks of 125 c. c. capacity were used. Fifty cubic centimeters of the honey-yeast-water-agar medium were put into each flask, after which the flasks were plugged and sterilized. Some of the yeast culture was transferred by means of a needle to the center of the hardened surface. Care was taken not to break the surface, for if this is done the medium cracks at this point on evaporation and ruins the giant colony.

Giant colonies of five strains of honey yeasts were grown very successfully in this way, and illustrations of these are found in Plates 1 and 2. The problem of maintaining uncontaminated colonies is reduced to a minimum by means of the flask cultures.

Three of the yeasts, strains 6, 7, and 8, were isolated from pollen which the bees had stored in the comb for brood food. Strain 9 was isolated from a sample of fermenting honey received from Hawaii. It belongs to the genus Zygosaccharomyces. Cultures of *Saccharomyces cerevisiae S. logos*, and *Z. priorianus* were also carried to see if there were any differences between certain known species and the honey yeasts, and were designated as strains 11, 12, and 13, respectively.

EXPLANATORY LEGEND FOR PLATE 1

A.—Morphology in young (*a*) and old (*b*) cultures of strain 1 which apparently corresponds with *Zygosaccharomyces mellis*, Fabian et Quinet.
B.—Two-month old giant colony of strain 1, enlarged 2 diameters.
C.—Morphology in young (*a*) and old (*b*) cultures of strain 2.
D.—Giant colony of strain 2, 2 months old, enlarged 2 diameters.
E.—Morphology in young (*a*) and old (*b*) cultures of strain 3.
F.—Giant colony of strain 3, 2 months old, enlarged 2 diameters.



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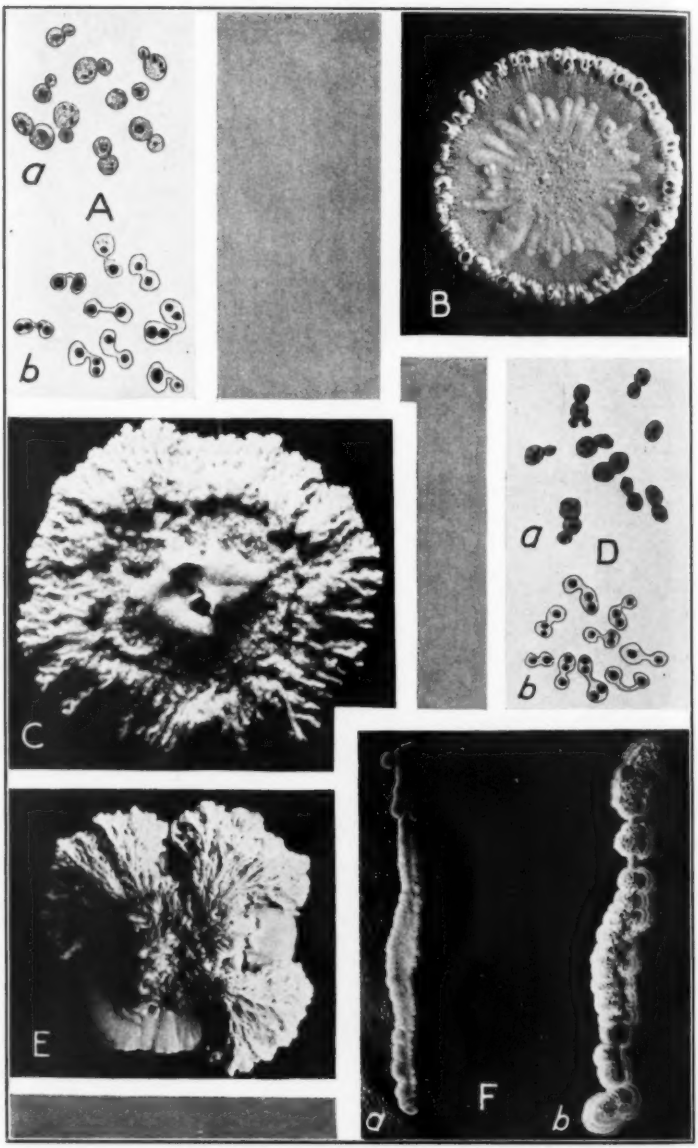

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RELATION BETWEEN CONCENTRATION OF HONEY AND INITIATION OF FERMENTATION

An experiment was set up to determine whether natural unheated honeys when diluted would undergo fermentation. Honey and sterilized water were mixed in small sterilized bottles in the following proportions:

Honey (parts by weight)	20	18	16	14	12	10
Water (parts by weight)	0	2	4	6	8	10

Seven Wisconsin honeys, all unheated and marketable, were used in this dilution experiment. One set of bottles from each of the seven honeys so diluted was inoculated by adding to each bottle a large loopful of a young vigorous growing culture of each of the five yeasts isolated from fermenting Wisconsin honey. A second set of bottles from each of the seven honeys so diluted was left uninoculated and kept as controls. Lead foil was wrapped around the top of each bottle to prevent evaporation. A strong fermentation was under way after 48 to 72 hours in the inoculated diluted samples. In the uninoculated controls fermentation was started in the more dilute samples after a week and in the more concentrated ones after two to three weeks. These results show that fermentation is promoted by a high percentage of water in the honey.

All of the diluted honeys which fermented were discarded and the undiluted ones were kept. As long as the honey remained liquid no noticeable fermentation took place, but after it had been stored a few months at room temperature, granulation began, and with this physical change in the honey signs of fermentation appeared in the inoculated samples.

A second series of samples of pasteurized honey was inoculated with five pure cultures of yeasts isolated from fermenting honey. As long as the honey remained liquid there were no signs of fermentation, but after a year and a half in storage the samples granulated and fermentation became apparent. Another series of samples of the same pasteurized honey was inoculated with small amounts of fermenting honey already granulated. These granules hastened crystallization in the samples and all fermented within a year. From this experiment it appears that crystallization of honey and fermentation are related to each other.

To study further this apparent relation of crystallization of honey to fermentation, moisture determinations were made on the syrup and crystal layers of honey. In the samples studied the moisture of the syrup layer was 9.11 per cent higher than that in the crystal layer, and the moisture was greater in the liquid layer than in the original honey.

When honey granulates, the water formerly serving as a solvent for the glucose crystals is retained in the liquid part of the honey and thus increases the moisture content of the syrup. Crystallization is

EXPLANATORY LEGEND FOR PLATE 2

A.—Morphology in young (*a*) and old (*b*) Zygosaccharomyces cultures of strain 4 which apparently corresponds with *Z. mellis*, Fabian et Quinet.
B.—Giant colony of strain 4, 2 months old; enlarged 2 diameters.
C.—Giant colony of strain 5, which is apparently the same as *Z. nussbaumeri*, Lochhead et Heron, 2 months old and enlarged slightly over 2 diameters.
D.—Morphology in young (*a*) and old (*b*) cultures of *Z. nussbaumeri*, Lochhead et Heron.
E.—Giant colony of *Z. nussbaumeri*, 1 month old, enlarged 2 diameters; the colony is smooth at first, later a rough secondary growth breaks out at the center and gradually covers the entire surface, as appears in the older colony at C.
F.—A streak culture of strain 3 (*a*) and *Z. barkeri* (*b*).

thus equivalent to diluting the uncrystallized honey with water. The syrup phase becomes dilute enough to permit the growth of yeasts that could not grow in the uncrystallized honey.

FERMENTATION CHARACTERISTICS OF HONEY YEAST

The fermentation reaction of the yeasts isolated from honey was studied in a medium containing about 2 gm. of sugar dissolved in 100 c. c. of 10 per cent yeast water. Ten cubic centimeter portions of this medium were placed in test tubes and sterilized, and each was inoculated with a loopful of a vigorous 4-day-old culture. These tubes were incubated at 28° C. and after seven days were analyzed for unfermented sugars by the method of Stiles, Peterson and Fred (*13*). By this time the medium in the tubes had ceased to produce gas and had begun to clear up. The results are given in Table 1.

From the table it is evident that there was some variation in the fermentability of the sugars used. Glucose, fructose, and mannose were easily fermented by the five strains of honey yeasts, while approximately one-third of the galactose was fermented, and lactose was very slightly utilized. A variation was noted in the fermentability of sucrose. Strains 1 and 4 fermented approximately only one-third while strain 2 utilized one-half. Strains 3 and 5 femented sucrose more energetically than did the other honey yeasts. With maltose, strains 1 and 4 utilized approximately the same amounts. Strain 5 fermented it energetically. Strain 2 fermented about one-third and strain 3 utilized over one-half.

TABLE 1.—*Fermentation of various sugars* [a] *by yeast strains isolated from honey and pollen*

Sugar	Percentage fermentation by strain No.—												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Glucose	98	97	97	99	96	96	96	97	97	95	97	99	98
Fructose	96	96	95	95	94	95	94	95	95	93	96	97	96
Mannose	96	93	95	96	93	93	94	96	95	90	96	96	96
Galactose	35	32	28	29	33	41	30	65	26	30	67	67	67
Sucrose	37	50	76	36	90	95	92	98	44	90	98	98	98
Maltose	58	30	55	60	81	98	96	18	58	65	71	60	55
Lactose	6	5	5	4	4	6	4	5	4	4	4	3	3

[a] Concentration of sugar before fermentation, about 2 per cent.

As the amount of raffinose, melezitose, soluble starch, and dextrin fermented could not be determined directly, the production of gas as indicated by the vaseline-seal method of Loewe and Strauss (*6*) was taken as a measure of fermentation. The yeasts isolated from fermenting honey did not form gas with these carbohydrates and apparently do not ferment them.

FERMENTATION PRODUCTS

METHODS OF ANALYSIS

In order to study the products of honey fermentation, 450 c. c. of 1 part honey and 1 part of 10 per cent yeast water were placed in liter flasks and sterilized. Small flasks containing 50 c. c. of the same medium were heavily inoculated with the honey yeasts from

slopes, and incubated. The fermenting material in the small flasks was used for inoculating the media in the large flasks. Sterile fermentation traps were inserted through corks and the whole waxed tightly into the mouth of the flask. Enough 20 per cent sulphuric acid solution was poured into the trap so that the opening of the tube leading from the interior of the flask was below the surface of the acid. The flasks were incubated at 28° C. After 40 days, when the production of gas had ceased and the material in the flasks had begun to clear, the liquid was analyzed for alcohol and acids.

ALCOHOL.—One hundred cubic centimeters of the honey medium, after fermentation was completed, were put into a distilling flask, a small amount of dry phenolphthalein was added, and the medium brought to neutrality. Next, 50 c. c. of distilled water, 5 gm. of NaCl, and a little tannic acid were added. The latter substance prevented frothing during distillation. One hundred cubic centimeters of distillate were collected, cooled to 15½° C., and the specific gravity determined by means of a pycnometer.

TITRATABLE ACID.—Fifty cubic centimeters of distilled water were added to 10 c. c. of the fermented medium and the mixture was brought to boiling. Phenolphthalein was used as an indicator and titration continued until the first permanent pink color appeared.

VOLATILE ACID.—Two hundred cubic centimeters of the fermented medium were acidified with 5 c. c. of N/5 sulphuric acid, distilled with steam, and 1,500 c. c. distillate collected. The distillate was brought to boiling and titrated with N/10 NaOH to the phenolphthalein end point.

NONVOLATILE ACIDS.—One hundred cubic centimeters of honey medium after fermentation was extracted for four days with ether. After the ether had been removed water was added and the extract titrated with N/10 barium hydroxide.

CARBON DIOXIDE.—The amount of carbon dioxide given off by each yeast during fermentation was determined by weighing the flask of fermenting honey at intervals. The flasks were sealed with fermentation traps and the carbon dioxide escaped through the sulphuric acid.

RESULTS OF ANALYSES

Carbon dioxide was measured quantitatively on 10 of the honey and pollen yeasts with the results that appear in Table 2. Strains 11, 12, and 13 failed to grow in a 50 per cent concentration of honey. From observations, fermentation in a 50 per cent honey medium is relatively rapid compared with what it would be in an ordinary sample of honey. In the case of the latter, it requires a long time for the fermentation to begin and it extends over a period of six months to a year. Evolution of carbon dioxide is very slow. In the 50 per cent honey medium the fermentation appeared to be over after 40 days and clearing was noticed. The evolution of carbon dioxide in 40 days varied from 3.0 to 6.3 gm. per 100 c. c. of 50 per cent honey for the 10 strains, as shown in Table 2.

In experimental fermentations of 50 per cent honey about equal quantities (4 to 5 gm. per 100 c. c.) of carbon dioxide and alcohol were produced in 40 days, as shown in Table 3. Small quantities of acid, mainly nonvolatile, were also produced. It was thought at first that on account of the large amounts of sugar present in the 50 per

cent honey medium, a depressing action was caused on the yeasts, which accounted for the small amounts of alcohol and acids formed. The concentration of honey was reduced to 20 per cent, and here again approximately the same amounts of alcohol and acid were formed. The acids and alcohol probably contribute the products which apparently cause the off-flavor of fermented or slightly fermented honey.

TABLE 2.—*Evolution of carbon dioxide from 50 per cent honey solution when fermented with 10 strains of yeast isolated from honey and pollen*

Duration of fermentation (days)	Grams of CO_2 evolved per 100 c. c. of honey when fermented with strain No.—									
	1	2	3	4	5	6	7	8	9	10
3	0.7	0.7	0.7	0.4	0.7	0.9	1.1	0.2	0.6	0.4
6	.9	.7	.9	.7	.7	1.1	1.6	.4	.6	.7
10	.7	1.3	.4	.4	.7	.9	.9	.2	.6	.7
13	.7	.4	.7	.7	.7	.7	.9	.2	.6	.4
20	1.1	1.1	.9	.9	1.1	.7	.7	.4	1.0	.9
27	.7	.7	.7	.7	1.1	.7	1.1	.7	1.0	.9
40		.7	.2	.2	.2			.9	.1	.1
Total	4.8	5.6	4.5	4.0	5.2	5.0	6.3	3.0	4.5	4.1

TABLE 3.—*Fermentation products of five typical honey yeasts produced in a 50 per cent honey fermentation lasting 40 days*

Yeast strain No.	Production per 100 c. c. of 50 per cent honey of—				
	Carbon dioxide	Alcohol	0.1 N acid		
			Titratable	Volatile	Nonvolatile
	Gram	*Gram*	*C. c.*	*C. c.*	*C. c.*
1	4.8	4.4	21	1.1	19.8
2	5.6	4.7	25	3.3	22.6
3	4.5	3.8	17	3.6	14.3
4	4.0	4.0	31	6.2	25.1
5	5.2	5.0	15	.3	15.0

MORPHOLOGICAL CHARACTERISTICS OF HONEY YEASTS

The published descriptions of species are frequently incomplete, but the writers have had the opportunity to compare their strains with cultures isolated by Fabian, formerly of Michigan, and Lochhead of Ottawa, Canada, who have been kind enough to send transfers of their honey yeasts. Cultures of *Zygosaccharomyses barkeri* and *Z. priorianus* have been obtained from Tanner of Illinois, Kluyver of Delft, Holland, and the American type culture collection in Chicago.

The morphological characters of the five yeasts isolated from fermented honey were studied from 96-hour-old streaks on 20 per cent honey yeast-water agar incubated at room termperature. As suggested by Lochhead, a concentrated honey gelatin was used for spore formation. This consisted of 100 c. c. of honey diluted with the same amount of water, to which was added 50 gm. of gelatin. Cultures were examined in liquid mounts of 0.1 N iodine. Vaseline was smeared around the cover slip and put over the drop of liquid to pre-

vent evaporation. Mounts made in this way are good for several days.

The strains were classified according to differences in (1) morphological characters of their giant colonies, (2) ring formation in flasks of fermenting wort, (3) type of growth on slopes, (4) appearance of cells under the microscope, and (5) their ability to ferment various sugars. The size of the cells of the different strains when grown on separate media is shown in Table 4:

TABLE 4.—*Relative size of cells of five yeast strains isolated from honey when grown on different media*

FROM STREAKS ON 20 PER CENT HONEY AGAR, 96 HOURS OLD, GROWN AT ROOM TEMPERATURE

Measurement	Size in μ of cells of yeast strain No. —				
	1	2*	3	4	5
Average length μ	5.59	9–4.6	4.16	5.35	4.66
Average width μ	3.94	2.75–3.44	3.71	3.8	3.8
GROWN IN 50 PER CENT HONEY 6 DAYS AT ROOM TEMPERATURE					
Average length μ	4.8	8.12–4.25	4.13	4.78	4.14
Average width μ	3.65	2.62–3.9	3.32	3.75	4

*Strain 2 persisted in having two types of cells, elongated and round.

DESCRIPTION OF YEASTS ISOLATED FROM FERMENTED HONEY

TYPE 1. (Pls. 1 and 2, A, B.)—Strains 1 and 4 form medium heavy rings in flasks of fermenting wort. Glucose, fructose, and mannose are fermented energetically, while galactose, sucrose, and maltose are fermented more weakly. Four-day-old cultures incubated at room temperature on 20 per cent honey agar show mostly elliptical cells, single or in groups of two and three. When a 50 per cent honey broth is used the cells are smaller. Conjugation and spore formation were observed on concentrated honey gelatin. The giant colonies of the two strains have not the same appearance, yet cultures have the same fermentative properties as type 3 of Fabian and Quinet and culture D1 of Lochhead and Heron, and so may be classed as *Zygosaccharomyces mellis* Fabian and Quinet.

TYPE 2. (Pl. 2, C, D, E.)—Strain 5 forms an extremely heavy ring in a flask of fermenting wort. Glucose, fructose, mannose, sucrose, and maltose are fermented strongly, while galactose is fermented more weakly. The cells are round, generally in pairs. The giant colony appears smooth at first with a folded and wrinkled secondary growth, as shown in Plate 2, E. This yeast corresponds with culture J7 of Lochhead and Heron, named by them *Zygosaccharomyces nussbaumeri*.

TYPE 3. (Pl. 1, C, D.)—Strain 2 forms medium heavy rings in flasks of fermenting wort. Glucose, fructose, and mannose are fermented energetically, while galactose and maltose are utilized to a less degree. This strain possesses cells of varying shapes, round or elliptical, and very much elongated. Elongated cells are always found attached to round ones, which gives the culture a characteristic

appearance. Conjugation preceding sporulation has been observed, but cells form spores without having undergone conjugation, only the beginning of a canal being noticed. This fact, which was not observed in the rest, and its different morphological and cultural characteristics on 20 per cent honey-agar medium entitle it to a separate classification. It appears, therefore, that the writers' strain 2 is not identical with culture D1 of Lochhead and Heron nor with type 3 of Fabian and Quinet. It does not appear to have been described before.

TYPE 4. (Pl. 1, E, F, Pl. 2, F.)—Strain 3 forms a very light ring in flasks of fermenting wort. Glucose, fructose, mannose, and sucrose are fermented strongly, while galactose and maltose are fermented more weakly. The cells of this yeast are mostly round, though some are elliptical. Usually two cells are joined, although they may appear singly. Conjugation between cells has been observed before spore formation on concentrated honey gelatin. This yeast has the same fermentative properties as type 2 of Fabian and Quinet and culture E6 of Lochhead and Heron and has been placed by them with the previously described *Zygosaccharomyces barkeri*. Pure cultures of *Z. barkeri* have been obtained from Professor Tanner of Illinois and from the American type culture collection. The type of growth of *Z. barkeri* from these sources on slants and in giant colonies is not in the least like that of the cultures of Fabian and Lochhead, which are the same as the writers' strain 3. In Plate 2, F, strain 3 is shown on the left and *Z. barkeri* on the right. It appears that strain 3 is a new species.

PREVENTION OF FERMENTATION IN HONEY

The writers have found it possible to prevent fermentation of honey by holding it at 100° F. for several months, 122° for 24 hours, or heating it to 160°. Nussbaumer (*9*) recommended heating honey to 70° C. for 30 minutes, and Fabian and Quinet (*2*) found that honey heated to 145° F. and held there for 30 minutes would not ferment. These last temperatures are rather difficult to maintain with the equipment found in the average commercial beekeeper's honey house. Honey kept at points above room temperature for a period of time has a tendency to darken in color and to lose some of its flavor.

If the honey is heated very quickly to 160° F., poured in containers, sealed while hot and cooled soon afterwards the color and the flavor will not be changed to any great extent. Fermentation, provided the honey has been heated all the way through to this temperature, is immediately and completely stopped. Granulation is delayed for some time, but when this honey does granulate the crystals are usually large. Honey which has not been heated should be held in storage at temperatures below 52°, for honey yeasts will not grow below this temperature.

SUMMARY

This paper reports the results of a study of yeasts isolated from fermenting honey.

It is shown that when honey granulates the water serving as a solvent for the glucose is retained in the liquid part of the honey, and increases the moisture content of the syrup. Crystallization is

thus equivalent to diluting the uncrystallized honey with water. The syrup becomes dilute enough to permit the growth of yeasts that could not grow in the unchanged honey.

The fermentation process in honey is slow, extending over a period of six months to several years. About equal quantities of carbon dioxide and alcohol (rarely over 5 per cent), together with small amounts of nonvolatile acids, form the chief fermentation products.

Some of the yeasts isolated from fermenting honey correspond with previously described species. What appear to be two new species are described in this paper.

Fermentation of honey can be prevented by heating the honey to 160° F., pailing it while hot, and cooling it immediately. Honey in storage will not ferment if kept at temperatures below 52° F.

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SOME PHASES OF PLANT DEVELOPMENT UNDER VITAGLASS [1]

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INTRODUCTION

Following the production of glasses with increased penetrability to shorter ultra-violet radiations there has naturally developed considerable interest in the possible function of these rays in plant life. This applies more particularly of course to sunlight, rather than to the shorter radiations emitted by ultra-violet lamps. With the discovery of activation of ergosterol to confer antirachitic properties upon foods, distinct possibilities have arisen that radiations of the middle ultra-violet region of the spectrum are of significance in plant metabolism. This is the limited region of sunlight to which ordinary glass is impermeable, extending approximately from 2,900 to 3,100 Ångström units (Å). The scanty literature in this field will be reviewed only briefly.

A rather comprehensive review of the effects upon plants of ultra-violet rays from artificial sources is given by Eltinge (*11*) [2] in connection with her anatomical observations in this field. As early as 1894 Bailey observed leaf injury in truck crops illuminated by a naked carbon arc. Several investigators have found similar injury to tissue structure, together with destruction of anthocyan pigments, in plants exposed to the mercury-vapor lamp. These results are only of passing interest here since they have been found to be due to radiations shorter in wave length than those reaching the earth's surface in sunlight.

Eltinge employed a Uviarc quartz lamp partly at a distance (250 cm.) from the plants, which greatly reduced injurious effects, and she further introduced screens of Quarzlite glass and Vitaglass. The former cut off radiations shorter than 3,130 Å, a value slightly greater than obtains with common window glasses. In the case of Vitaglass the lower limit of transmission was 2,890 Å, or slightly lower than the minimum wave length found in sunlight. Use of the lamp increased by 30 seconds daily from an initial period of this length. This treatment introduced essentially moderate increases in that portion of the spectrum between yellow and middle ultra-violet. The general trend of results indicates responses to the added radiation in vegetative expansion or time of flowering, which varied with the species. Maize, for example, was stimulated in growth while radish was depressed. These effects were more marked under the enhanced ultra-violet exposure with Vitaglass. Here Eltinge found enlargement of cells in the leaf blade and of fibrovascular bundles in the stem associated with increased thickness of these organs. No change in the hydrogen-ion concentration of the sap or decomposition of chlorophyll could be detected in response to [increased] ultra-violet radiation.

[1] Received for publication Feb. 18, 1931; issued August, 1931.
[2] Reference is made by number (italic) to Literature Cited, p. 161.

Decrease of starch in the stem of coleus and increase in the bean stalk followed this treatment. There was generally a slight decline in percentage of ash in the dry matter.

The present investigation is concerned with the elimination of a small portion of ultra-violet (about 3,100 to 2,900 Å) in sunlight by the screening effect of common glass. As early as 1918 Schanz (*22*, *23*) used Euphos glass to eliminate solar rays below the visible range, and concluded that long ultra-violet rays were generally unfavorable to plant development. However, there was considerable lack of adequate control of environmental factors in this pioneering investigation. The more recent work of Popp (*19*) is relatively more significant. He utilized glasses of special transmitting capacity in the roofs of separated greenhouse sections. The total intensity of illumination varied considerably under these different glasses, but apparently was not low enough in any case to become a limiting factor. All other factors except the quality of light were kept relatively uniform in value. Anatomical observations and chemical analyses of the tissue in tests with several species, showed little effect due to elimination of ultra-violet rays, other than a slight elongation of stems. The differences between common glass and one resembling Vitaglass (G86B) were considered negligible. These results were obtained in the period from September to November, inclusive. Using the same equipment, Pfeiffer (*18*) found in tests with oats, sunflower, four-o'clock, and soybean, declines in thickness and differentiation of leaf, height and diameter of stem, and vascular development with elimination of ultra-violet from sunlight. Despite Popp's conclusion as to the dispensability of ultra-violet rays, the writers will consider later certain respects in which his results indicate possible significance for this fraction of solar radiation.

Reinhold and Schulz (*21*) operated with well-ventilated hotbeds protected from cross illumination by the different glass in adjacent window frames. It is stated that the same temperature was maintained under all of the glasses. Tests with several species in the spring and summer gave generally differences of yield from exclusion of ultra-violet rays not exceeding the limits of experimental error. However, enhanced yields of lettuce and dwarf bean and a decline with melon (apparently cantaloupe) exceeded these limits. In a test of the rôle of phase of growth, elimination of ultra-violet rays delayed the ripening of tomatoes but not of other species. The conclusions of these investigators, favorable to the dispensability of ultra-violet rays, agree with those of Popp, but here also the data appear to admit of reservations. More favorable conclusions were drawn by Dix (*10*) and Tincker (*25*) from limited results. Dix obtained with wheat and cabbage increased yields of more succulent tissue under a glass resembling Vitaglass than under common glass. Tincker recovered larger roots of radish and carrot under Vioray glass and Vitaglass than under common glass, but lettuce and cucumber he found to be unaffected.

Some of the more extensive investigations here cited are unfavorable in their general conclusions as to appreciable significance in plant growth of the region of shorter ultra-violet in sunlight. However, even here certain irregularities in results indicate that plant species respond differently to this environmental factor. This situation seems to justify further investigation of the problem.

PROCEDURE

With minor exceptions, as will later be noted, the general procedure involved rearing plants in flats or deeper boxes of soil, according to requirements. These plantings were cared for in common with other operations of the greenhouse ranges.[3] Vitaglass was used for six tiers on both slopes of a greenhouse roof. Such areas, directly opposite one another, did not include ventilators and the panes abutting gable and gutters. The house occupied the common north-to-south position, which is not the most advantageous for light transmission between late fall and early spring. As a result of the fact that this illumination treatment was located at the north end of the house the considerable infra-red transmission of Vitaglass as com-

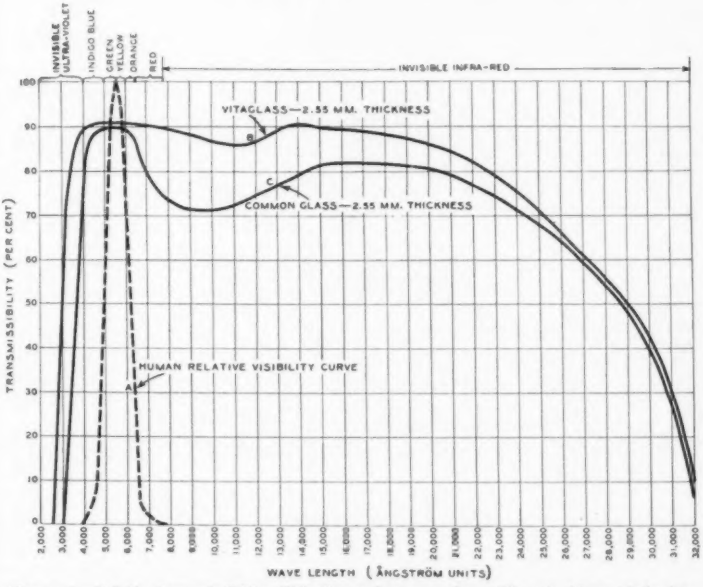

FIGURE 1.—Relative transmissibility of Vitaglass and common glass. The actual transmission by common glass in the present work was somewhat less than that shown because the glass actually used was thicker than indicated in the figure

pared with common glass was augmented by the excess heating capacity of the radiator intakes. Partial compensation for this effect was secured by the use of an oscillating fan, directing currents from the latter off the north wall of the house at an elevation of about 6 feet. In this way a temperature difference of about 2° C. between the bench surfaces occupied by these plantings was reduced to about 1°. The relative transmission of common glass and Vitaglass is shown in Figure 1, while the effect of ventilation upon temperature records taken at the surface of the greenhouse benches appears in Figure 2. Except when use of the fan was discontinued, as in Novem-

[3] Acknowledgment is due W. Newell, foreman, and M. Kelhoefer for faithful assistance in these operations.

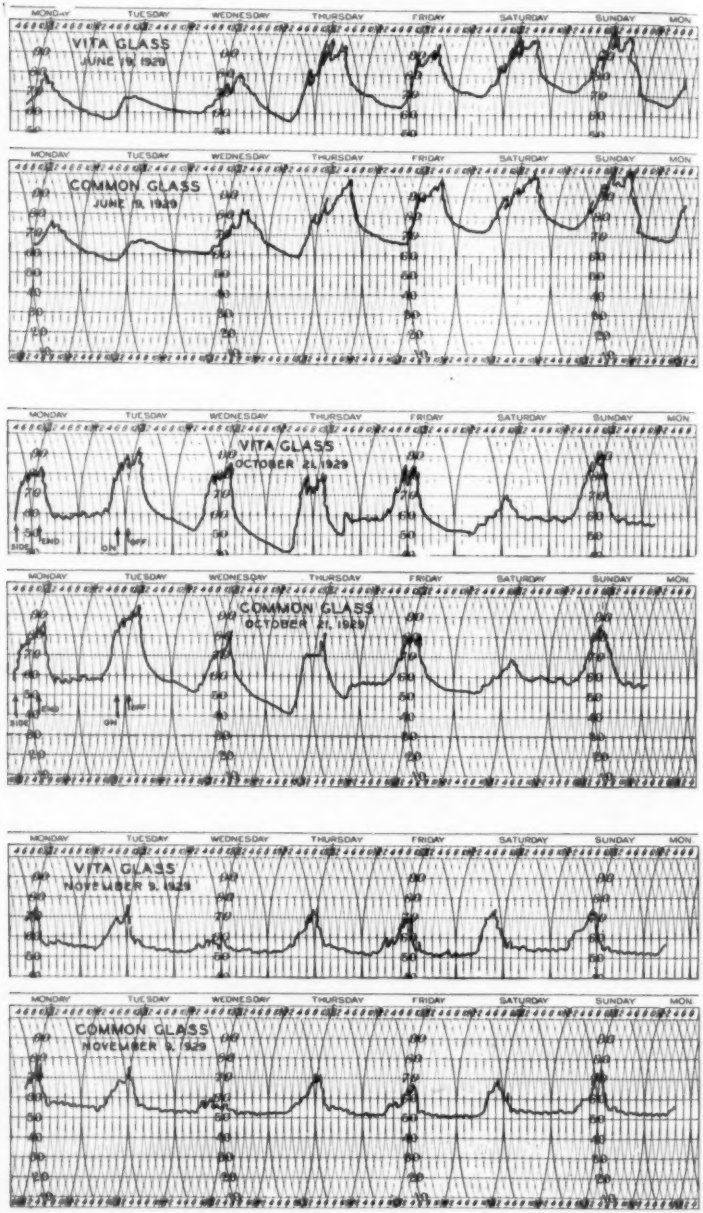

FIGURE 2.—Specimen thermograph records (degrees C.) under Vitaglass and common glass. The arrows indicate differences of fan placement within the greenhouse and in its use

ber, the midday temperature balance was not invariably in favor of cultures under Vitaglass.

In the summers of 1929 and 1930 the Vitaglass area was dressed with limewash, in common with the rest of the roof. It was supposed that this treatment might favor the functioning of this glass (*8*), but it is possible that attack of the applied alkali modified its transparency. Pending further information on this point, the dressing practice has been discontinued. Attention should be called to the fact that antirachitic efficiency is retained by weathered and aged Vitaglass (*7*). The writers' observations indicate that its potency in plant development is similarly prolonged.

The present phase of this investigation embraces only a general survey of developmental differences and variations in grosser chemical composition of plants reared under common glass and Vitaglass. Accordingly, a considerable range of species has been tested, including those evaluated for vegetative, flowering, and fruiting performance. It was anticipated that this treatment might disclose species meriting more exhaustive consideration.

As to the procedure in chemical examination of the tissues, the general recommendations published elsewhere have been observed (*1*, *2*, *3*, *4*, *5*).

SPECIAL TREATMENT AND RESULTS

GRAMINEAE

WHEAT (TRITICUM SATIVUM)

Preliminary tests with wheat were made in an outdoor frame. This was so constructed, with sloping glass roof and sheet-metal partitions, as to provide a southern exposure and confine the light received by each group of cultures to the transmission of its respective sash. One compartment was covered by Vitaglass and two others by common glass. For about three hours on each fair day one of the common windows was replaced by an empty sash to permit direct exposure to midday solar radiation. During this period the temperature upon the soil surface was about 2° C. lower in the open cultures than in the covered ones.

Marquis wheat of pure-line selection was sown in cubical boxes containing 15 kgm. of rich silt loam. The water content of the soil was regulated to optimal planes and the stand was reduced to 20 plants per box. In a test conducted from October 8 to November 18, 1927, it was considered advisable, on account of severely cold weather, to transfer the cultures on November 5 to a greenhouse regulated at about 15° C. Thereafter it was only possible to return the plants to the usual midday outdoor setting on four scattered dates. There was considerable variation in yield and nitrogen content in individual cultures. While these values were lower in the controls than in cultures exposed under the glasses, there were no distinct differences under the two forms of glass. It must be admitted, however, that the low proportions of ultra-violet in sunlight at this late season, together with the enforced greenhouse protection, were unfavorable to differentiation of radiation effects.

A second test was conducted from April 13 to May 30, 1928, a period obviously much more propitious for effectiveness of ultra-violet radiation than the earlier one. The results appear in Table 1.

TABLE 1.—*Effect of glass light screens upon the growth and nitrogen content of Marquis wheat plants following exposure to solar rays from April 13 to May 30, 1928*

Light screen used	Culture No.	Plant measurements			Weight of air-dry matter	Nitrogen content of dry matter
		Length over all	Height to top node	Diameter of bottom internode		
		Centimeters	*Centimeters*	*Millimeters*	*Grams*	*Per cent*
None	1	40.0	18.8	2.6	6.5	5.7
	2	40.5	15.0	2.9	7.9	4.9
Vitaglass	3	42.5	18.8	2.9	8.8	4.7
	4	43.7	18.8	3.2	6.9	4.9
Common glass	5	46.3	20.0	3.1	7.0	4.2
	6	47.5	17.5	2.9	6.8	4.6

These data show considerable irregularity of performance in individual plantings, with general overlapping of results from different radiation treatments. In the single case of leaf length (length over all less stem height) consistent differences appear, indicating a suppressing effect of solar rays, which are more completely screened out by common glass than by Vitaglass. The value of this function was 14 per cent less, or 8 per cent in terms of total length, under the Vitaglass.

Further tests with wheat were made in the greenhouse equipped with Vitaglass. On May 1, 1929, 25 days after seeds of the Marquis variety had been sown in flats of composted loam, the plantings were thinned one-half, a distance of about 1.5 cm. being left between plants. Data obtained on the harvested plants are presented in Table 2.

TABLE 2.—*Effect of glass light screens upon the growth and composition of Marquis wheat plants sampled on May 1, 1929, 25 days after sowing*

Screen	Plant length over all	Proportion of dry matter	Yield of dry matter	Partial composition of dry matter		
				Ether extract	True protein	Nonlipide, nonprotein nitrogen
	Centimeters	*Per cent*	*Grams*	*Per cent*	*Per cent*	*Per cent*
Vitaglass	32.5	10.5	26.6	5.9	18.9	0.85
Common glass	35.0	9.5	25.7	5.7	21.3	.83

The length of the plants was about 7 per cent less under Vitaglass than under common glass, and the fresh weight was likewise less. The percentage of protein in the dry matter produced under Vitaglass was about 11 per cent less than the percentage of the dry matter produced under common glass. In other respects the differences in the plantings were not beyond the range of expected variability.

On May 1 an unthinned flat from beneath each type of glass was placed out of doors in a coldframe to test the resistance of the plants to cold. Frosts occurred on May 3 and 4 at 0° C., and slight injury was subsequently observed as darkening and withering of leaf tips in both flats. These plants were kept out of doors and adequately watered. On June 6 they appeared as shown in Figure 3. The plants previously exposed under Vitaglass are distinguished by dark

color and erect posture, as well as limited stooling. As the plants were now heading out, the flats were returned to their respective greenhouse positions in order to protect the seed against birds. At this time they received light applications of KNO_3. Despite the fact that soil moisture was permitted to decline, a few plants were green under common glass when harvested on July 12, but only normal ripe grain was analyzed. The data of this crop are presented in Table 3. These show decreased yields under Vitaglass, which

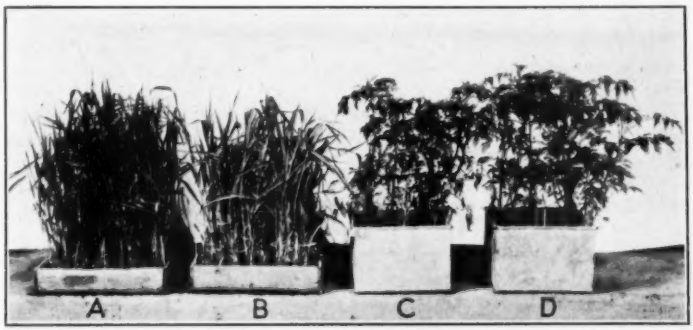

FIGURE 3.—Plants tested for cold hardiness at three weeks of age; grown under glass. Photographed June 6, 1929, after five weeks' outdoor exposure. A and C are Marquis spring wheat and Globe tomato that were started under Vitaglass, B and D the same, respectively, started under common glass

might be due to injury from cold, but no essential difference in composition.

TABLE 3.—*Effect of glass light screens upon the yield and partial composition at maturity (July 12, 1929) of Marquis wheat plants following outdoor exposure for hardiness test*

Screen	Yield of air-dry straw	Hemicellulose of dry straw	Crude fiber of dry straw	Yield of cured grain	Crude protein of dry grain
	Grams	*Per cent*	*Per cent*	*Grams*	*Per cent*
Vitaglass	41.4	44.7	42.2	[a] 15.6	18.7
Common glass	52.0	45.0	42.3	[b] 23.4	17.1

[a] 57 bearing heads. [b] 53 bearing heads.

The plants kept continuously under glass displayed on May 21 essentially the same differences seen in Figure 3. At this time the plants were a little longer and considerably thicker of stem under common glass. A darker color of leaf and bluish bloom on the stem distinguished the plants under Vitaglass at this time, but the bloom developed about two weeks later under common glass. Heads emerged four days earlier under Vitaglass than under common glass, and the stems of the plants under Vitaglass were about 10 per cent longer than those under common glass. When harvested on July 5 there were still a few immature plants under common glass, so that there were selected for purposes of comparison 100 of the better developed plants under each type of glass. These gave the data assembled in Table 4.

TABLE 4.—*Effect of glass light screens upon the yield and partial composition of Marquis wheat plants harvested July 5, 1929, after continuous growth under glass*

Screen	Yield of air-dry straw	Crude protein in straw	Yield of dry grain	Weight per seed	Ether extract	True protein	Nonlipide, nonprotein nitrogen
	Grams	*Per cent*	*Grams*	*Mgm.*	*Per cent*	*Per cent*	*Per cent*
Vitaglass	84.8	3.9	78.5	32	2.2	16.4	0.61
Common glass	102.9	4.0	68.8	34	2.3	17.2	.65

It appears that there was increased seed production at the expense of straw under Vitaglass, but without modification of grosser composition in the tissues.

It seemed desirable to test the relative vitality of seeds produced under the two types of glass. Accordingly, seeds recovered the previous summer under each glass treatment were sown under both types of glass on September 28, 1929. The cultures were thinned on December 6, and to each flat was applied a solution containing 0.35 gm. KNO_3 and 0.18 gm. $Ca(H_2PO_4)_2$. The plants produced from seed reared under common glass gave the results in Table 5.

TABLE 5.—*Effect of glass light screens upon the yield and partial composition of second-generation Marquis wheat plants grown under glass, December 6, 1929, 69 days after sowing*

Screen	Yield of dry matter	Ether extract	True protein	Nonlipide, nonprotein nitrogen
	Grams	*Per cent*	*Per cent*	*Per cent*
Vitaglass	[a] 16.2	6.3	16.6	1.00
Common glass	[b] 15.9	5.5	16.6	.97

[a] 37 plants. [b] 39 plants.

The only significant difference here is the higher content of ether extract under Vitaglass.

As the remaining crop matured, heads appeared under common glass on February 17 and under Vitaglass 10 days later. To promote seed development under the relatively crowded conditions, there were applied on February 20, 125 mgm. per flat each of KCl and $Ca(H_2PO_4)_2$. On June 2 the crop produced from seed reared under common glass was harvested, and two days later that from seed reared under Vitaglass was also harvested, a few plants being still immature. Only the total nitrogen content of the dried grain was here determined, the values obtained ranging from 3.34 to 3.41 per cent. In this respect there was no difference in seed produced by plants which underwent a period of semidormancy due to the low solar radiation of winter.

Simultaneous with the Marquis wheat experiments just described, tests of winter varieties were made. The varieties included were Minhardi, Kanred, and Nebraska 28. The appearance of the seedlings at the date of thinning is shown in Figure 4, a more erect habit of growth characterizes the hardier varieties under Vitaglass. Data regarding the Minhardi variety at this time are given in Table 6. There are found here only such variations as appear to be within the

limits of ordinary variability, with the possible exception of a greater proportion of nonprotein nitrogen. The yields of Nebraska 28 and Kanred varieties were considerably greater under Vitaglass than under common glass.

TABLE 6.—*Effect of glass light screens upon the yield and partial composition of Minhardi wheat, December 6, 1929, 69 days after sowing*

Screen	Yield of dry matter	Ether extract	True protein	Nonlipide, nonprotein nitrogen
	Grams	*Per cent*	*Per cent*	*Per cent*
Vitaglass	[a] 9.8	6.6	17.1	0.99
Common glass	[b] 10.2	6.3	20.2	.67

[a] 36 plants. [b] 35 plants.

These various cultures were continued to maturity and the plants were harvested in late May to early June. Fertilizer applications were made as to Marquis. Only the ether extract and true protein

FIGURE 4.—Winter wheat plants photographed December 5, 1929, after nine weeks' continuous exposure under glass: A, C, and E depict Minhardi, Kanred, and Nebraska 28 varieties grown under common glass; B, D, and F, the same varieties, respectively, grown under Vitaglass

content of the grain were determined, and these were essentially equal for the two glass treatments. Thus, the winter wheats gave no evidence of response to Vitaglass treatment after a prolonged semidormant period of growth.

MAIZE (ZEA MAYS)

On March 30, 1930, the Golden Glow variety of maize was planted in flats of soil. The seed came from the general run of experiment station breeding fields. One-half (eight) of the plants were removed from three flats of soil under each type of glass on May 1. The yields of air-dry tissue were 68.5 gm. under Vitaglass and 57.6 gm. under common glass, representing percentages of 7.5 and 6.7, respectively, of the fresh plants. There was therefore a somewhat greater yield of dry tissue under Vitaglass.

At the time of this harvest an unthinned flat from beneath each type of glass was exposed out of doors for a cold-hardiness test. Immediate and severe injury was apparent in both cases. The effect seemed at first somewhat greater on the plants from beneath common glass, but in two weeks both cultures were equally and apparently irrecoverably affected. However, on June 20 renewed growth was apparent, particularly in the culture formerly confined under common glass.

When further thinned on May 10, the plants which had remained in the greenhouse were somewhat taller and greener under Vitaglass than under common glass. Tassels and silks appeared in early June, two days earlier under Vitaglass than under common glass. To promote maturation the cultures were reduced to two plants each on June 15 and received an application of KNO_3. The crop under Vitaglass was harvested July 12 and that under common glass six days later, both curing for one week in the greenhouse. From the crop under Vitaglass 7 mature ears and 3 unproductive ones were taken while only 6 small, poorly filled ears were produced under common glass. Three internodes adjacent to the best ear were taken from each stalk. The average weight of the section was 32.5 gm. under Vitaglass and 25.8 gm. under common glass, while the average weight of acceptable ears was 55.6 and 40.9 gm., respectively. Thus the plants were considerably larger under Vitaglass. In Table 7 are assembled the analytical data obtained from young plants and ripened grain. It appears that there were considerably greater proportions of ether extract and protein in the young plants under Vitaglass, but that these differences did not carry over into the seed to a significant extent.

TABLE 7.—*Effect of glass light screens upon the partial composition of maize plants harvested May 10, after 41 days' growth under glass, and of mature grain harvested July 12 (Vitaglass) and July 18 (common glass), 1929*

Screen	Percentage composition of—					
	Plants			Grain		
	Ether extract	True protein	Nonlipide, nonprotein nitrogen	Ether extract	Crude protein	Nonlipide, nonprotein nitrogen
Vitaglass	4.3	9.1	0.36	4.2	9.5	0.18
Common glass	3.2	5.6	.63	3.9	8.9	.18

Possible cumulative effects were tested also in this crop by planting on September 25, 1929, seed produced under both types of glass the preceding spring, as well as some of the original supply. By October 10 it was apparent that plants of the second generation lacked vitality. This was more noticeable in the case of seed from Vitaglass treatment. Plants taken in thinning the cultures on November 9 gave the data shown in Table 8.

TABLE 8.—*Effect of glass light screens upon the yield and partial composition of second-generation maize plants sampled November 9, 1929, 45 days after planting*

Item	Composition of plants from seed of—					
	Original plants		Plants grown under Vitaglass		Plants grown under common glass	
	Vitaglass	Common glass	Vitaglass	Common glass	Vitaglass	Common glass
Dry weight per plant, grams	2.3	1.7	(a)	[b] 3.4	2.6	2.0
Crude protein per cent	29.8	26.9	----------	27.2	28.6	24.6

[a] No plants available.

[b] Average of only 3 plants.

At this date the stand was reduced from a maximum of 12 per 15-kgm. box of soil to four each, with the single exception noted in the table. It appears that the plants were generally larger and contained more crude protein under Vitaglass.

On December 6 the stand was reduced to two plants per box, these receiving an application of 0.35 gm. KNO_3 and 0.18 gm. $Ca(H_2PO_4)_2$. Tasseling and silking began soon after this, but about six days earlier under Vitaglass than under common glass. Despite artificial pollination to compensate for irregularity of development of silks, the crop was sterile with the exception of one plant from the original seed under Vitaglass. The stover harvested on January 30, 1930, showed no essential difference in nitrogen content under the two forms of glass. Thus there was in this respect no residual effect from earlier exposure to higher planes of ultra-violet light.

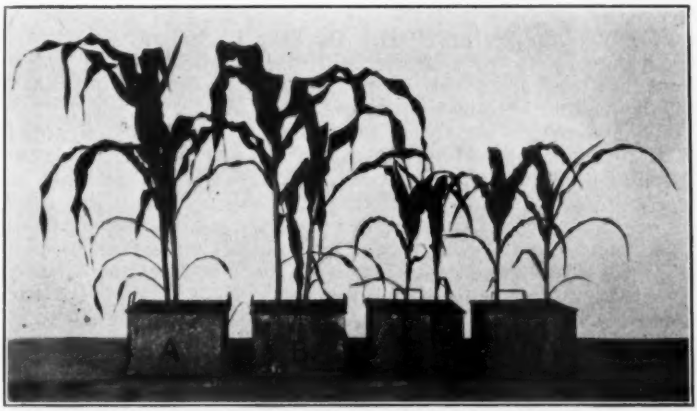

FIGURE 5.—Corn plants photographed April 1, 1930, at seven weeks of age, after continuous exposure under glass: A and B are Golden Glow from seed produced under Vitaglass, A having grown under common glass and B under Vitaglass; C and D are a pure-line selection from Illinois cold resistant, C having grown under common glass and D under Vitaglass

A further planting of maize in the same boxes was made on February 3, 1930, and on May 7 the plants were thinned to two per box. Some of the seed used was of a pure-line cold-resistant type developed by the Illinois experiment station and the United States Department of Agriculture. This was introduced to contribute greater uniformity of plant performance. The remaining seed was from the same source as that used in the previous test. Injury which developed from fumigation with tobacco smoke on February 27 indicated that the plants under Vitaglass possessed more open, succulent tissue than the others. Figure 5 shows the appearance of the plants on April 1. Tassels appeared under Vitaglass on April 12 and two days later under common glass, in the Golden Glow variety. Harvesting of the crop extended from June 4 in the case of plants from seed reared under Vitaglass to June 18 in the case of pure-line seed under common glass, the remaining plants being taken on June 7. Again fertilization had been quite irregular, so that only partial data were taken. These must be discounted because of the small numbers involved of a

species relatively variable in performance. The yield of air-dry stover from pure-line seed was 18 per cent greater under common glass despite the larger size of the other earlier plants. The yield from seed reared under common glass was 48 per cent greater under Vitaglass. In the case of grain produced by the latter plants both the ether extract and the true protein content were higher under Vitaglass than under common glass, the respective percentages being 4.7 and 4.2 for ether extract and 9.9 and 8.8 for protein. The results are indefinite as to yields but favor some carry-over of lipide differences from the foliage (as previously observed) to the seed.

SOLANACEAE

Tomato (Lycopersicum esculentum)

Seedlings of the Globe variety of tomato reared under common glass were transplanted to the cubical boxes of silt loam under both types of glass on April 6, 1929. On May 1 one-half (eight) of the plants were taken for analysis, with the results given in Table 9. These show that the plants were larger under Vitaglass and that the content of ether extract was greater.

At this time one box from each radiation treatment was exposed out of doors for the cold-hardiness test. Both cultures were severely injured at minimum air temperatures of about 0° C., but that from beneath Vitaglass more so. Figure 3, C and D, shows the appearance of these plants after continued exposure out of doors up to June 6. In the spring of 1930 a second collection of young tomato plants was prepared for the outdoor-hardiness test but the weather proved unfavorable. They were therefore placed in the ice can of a refrigerating machine. Four hours later when they were removed, the temperature about the plant tops was 0° C. After standing in a cool basement for 20 hours, the plants reared under Vitaglass gave evidence of serious frost injury.

Table 9.—*Effect of glass light screens upon the yield and partial composition of young tomato plants, May 1, after selective light exposure for 25 days*

Screen	Fresh weight	Ether extract	True protein	Nonlipide, nonprotein nitrogen	Hemicellulose	Crude fiber
	Grams	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Vitaglass	652	1.8	19.7	2.0	36.7	13.6
Common glass	510	1.3	18.9	2.0	37.8	13.9

On May 10 the stand was reduced to three plants per box. The average air-dry weight per plant removed was 2.4 gm. under common glass and 1.4 gm. under Vitaglass. There were now left three boxes, nine plants in each, those under Vitaglass being somewhat the paler. By May 31 a few fruits had attained a diameter of about 2.5 cm. under both glasses, but their average size was considerably greater under common glass on June 11. The vines showed also in this case the onset of maturation by the basal dying. One would expect the opposite effect to be produced if more heat was absorbed by the plants under Vitaglass. A light application of KNO_3 was made on June 20. Blossom-end rot soon became rather common and fruit so affected was discarded after it was weighed, as the ripening crop was

gathered. On July 10 there were left on the vines under Vitaglass 17 fruits of or above egg size and many smaller ones, while under common glass there were 28 larger green fruits and but few smaller ones. This might also be interpreted as signifying delayed maturation under Vitaglass, but the fruit actually colored off earlier under Vitaglass. On July 20 the final picking removed all but very small green fruits. Four ripe fruits from beneath each glass were preserved for analysis by slicing into a sufficient volume of boiling alcohol to give about 70 per cent concentration. The remaining fruits had been sliced and oven-dried as they ripened, killing at 98° C. and finishing at 70°. Sections about 20 cm. long were taken from the middle of the stems for analysis. Under Vitaglass 10 green fruits weighed 460 gm. and 27 ripe ones 2,375 gm., while the corresponding values under common glass were 644 gm. for 12 green fruits and 3,776 for 53 ripe ones. There was thus the greater set of fruit under common glass. Naturally, the average size per ripe fruit would be greater under Vitaglass than under common glass, because there was less crowding; the respective values were 88 and 71 gm. Sugars and acid were determined on the preserved fruit, the latter being only approximate in value, due to titration by indicators in a colored extract. The remaining values were obtained from dried tissue. These various data are assembled in Table 10.

TABLE 10.—*Effect of glass light screens upon the partial composition of tomatoes grown from April to July, 1929, under glass*

Screen	Percentage composition of ripe fruit					
	Dry matter	Ether extract	Reducing sugars	Acids, as malic	True protein	Nonlipide, nonprotein nitrogen
Vitaglass	5.3	1.4	13.3	6.6	4.6	0.97
Common glass	3.5	1.4	12.2	5.3	4.8	.71

An inspection of these values shows that the less succulent fruit was produced under Vitaglass. Computation on this basis gives nearly equal yields of dry matter in the ripe fruit, or 126 gm. under Vitaglass and 132 gm. under common glass. There is some evidence, in the higher proportions of simpler nitrogen compounds, acids, and perhaps sugars, that the fruit under Vitaglass was the seat of the greater metabolic activity. The mature stems show no essential difference in proportions of skeletal compounds.

A test for cumulative effects was made with this species by sowing on September 27 seed recovered under both types of glass the previous summer. The seedlings were transplanted to flats under the different glasses on October 16 and transferred to the cubical boxes of loam on November 9. Analysis of the tops of residual plants at this time gave the following values for crude protein: Seed from Vitaglass under Vitaglass 19.3 per cent, under common glass 17.4 per cent; seed from common glass under Vitaglass 19.2 per cent, under common glass 17.1. It thus appears that there is a considerable spread in crude protein content in favor of Vitaglass, irrespective of radiation treatment in the previous generation.

On December 5 the plants under Vitaglass displayed slightly greater height and considerably greater diameter of stem, as shown

in Figure 6. This might be either an effect of increased infra-red (heat effect) or a growth-stimulating effect of increased ultra-violet radiation. At this time a fertilizer application was made of 0.35 gm. KNO_3 and 0.17 gm. $Ca(H_2PO_4)_2$ per box. Blossoms appeared first under common glass on December 20, but two weeks later there were numerous buds under both types of glass. On January 12, 1930, the

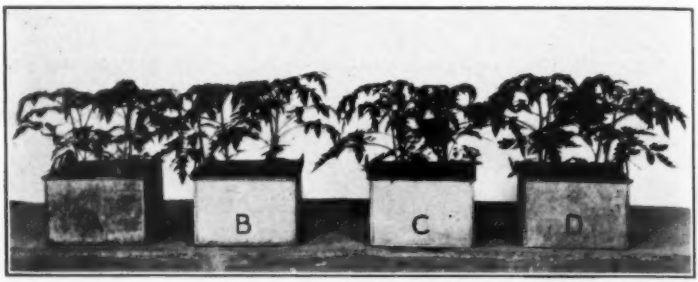

FIGURE 6.—Globe tomato plants photographed December 5, 1929, at 10 weeks of age, after continuous exposure under glass: A and B were reared from seed produced under common glass, C and D from seed produced under Vitaglass; A and C were grown under common and B and D under Vitaglass

two smaller plants in each box were harvested. These provided the data given in Table 11.

TABLE 11.—*Effect of glass light screens upon the yield and partial composition of tomato plants grown under glass, January 12, 1930, 107 days after sowing*

Screen	Yield from—		Ether extract	True protein	Non lipide, non protein nitrogen
	Vita seed	Common seed			
	Grams	*Grams*	*Per cent*	*Per cent*	*Per cent*
Vitaglass	47.2	50.4	4.1	12.2	0.67
Commonglass	36.6	37.8	4.0	13.0	.64

As regards yields, the behavior of duplicate cultures was consistent and there was pronounced superiority of Vitaglass, regardless of treatment in the preceding generation. As would be anticipated on genetic grounds, the effect is therefore purely an environmental one. Earlier differences of composition had disappeared at this time.

Artificial pollination was discontinued on February 10, when a total of 33 blossoms had set under Vitaglass and 24 under common glass. E. F. Burk, assistant in horticulture, called attention on this date to the generally shorter length of pistil under Vitaglass, which would favor fertilization. Although there had been little difference in the date of earliest blooming, the fruit ripened somewhat more rapidly under Vitaglass. The total yield in this instance was 59 ripe or large green fruits weighing 4,992 gm. and 48 fruits under common glass weighing 3,477 gm. Thus, despite a more crowded fruit population, individual fruits attained a greater average size under Vitaglass than under common glass, with values of 85 and 72 gm., respectively. When the vines were harvested on April 5 seven small fruits were removed under Vitaglass and 13 under common glass. The yield of air-dry

vines was nearly equal under the two glasses, being 484 gm. under Vitaglass and 507 under common glass.

Analytical results from composite samples of ripe fruit under each glass are given in Table 12. More succulent fruit was produced under Vitaglass. The difference from the earlier crop might follow soil moisture differences. These fruit solids were characterized by greater proportions of lipides and acids than under common glass.

TABLE 12.—*Effect of glass light screens upon the partial composition of ripe tomato fruits produced under glass, February 28 to April 7, 1930*

Screen	Dry matter [a]	Ether extract	True protein	Nonlipide, nonprotein nitrogen	Acidity of 1:100 extract	Acidity, as malic
	Per cent	*Per cent*	*Per cent*	*Per cent*	*pH*	*Per cent*
Vitaglass	4.0	1.8	4.1	0.68	4.40	[b] 5.37
Common glass	6.3	1.3	3.9	.81	4.85	4.26

[a] On this basis the total dry matter production in both ripe and larger green fruits would be 200 gm. under Vitaglass and 219 gm. under common glass.
[b] By potentiometric titration.

POTATO (SOLANUM TUBEROSUM)

Only one test was made with potato, the Irish Cobbler variety being used. Small eyepieces of nearly equal size were planted in 20-kgm. portions of loam soil contained in cubical boxes. The sprouts emerged on February 10, 1930, and on February 27 the lower internodes were twice as long under common glass as under Vitaglass. A month later the tops were more recumbent under common glass and the leaves were somewhat smaller than under Vitaglass. The vines also matured earlier under this treatment, so that the tubers were harvested on May 12 as compared with May 24 under Vitaglass. In comparing the data presented in Table 13 it must be recognized that they were derived from single plants. Aside from increased efficiency of the vines in producing tubers there is no significant difference related to radiation treatment.

TABLE 13.—*Effect of glass light screens upon the yield and partial composition of potatoes grown under glass and harvested in the middle of May, 1930*

Screen	Yield of air-dry vines	Yield of air-dry tubers	Dry matter in tubers	Ether extract in tubers	True protein in tubers	Nonlipide, nonprotein nitrogen in tubers
	Grams	*Grams*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Vitaglass	8.2	57.9	17.6	0.4	7.7	1.3
Common glass	13.5	54.9	16.5	.4	8.1	1.1

CRUCIFERAE

CABBAGE (BRASSICA OLERACEA)

Cabbage seedlings of the variety All Seasons reared under common glass were transplanted to the smaller cubical boxes of soil on April 6, 1929. On May 2 a culture from each radiation treatment was exposed in the coldframe as described in earlier tests. Three days later incipient injury was discernible on the leaf edges of plants which had been under Vitaglass. These cultures were left out of doors, and

on June 6 presented the appearance shown in Figure 7, with more coloring and with dying of the basal leaves in the now stunted plants which began growth under Vitaglass. Both cultures were returned to their respective exposures in the greenhouse on June 15, one-half (four) of the plants from each culture having been removed to promote heading. It is apparent that the plants under Vitaglass received a greater shock from frost than did the others. At this time they contained 14.5 per cent crude protein in the dry matter as compared with 13.8 per cent in the others.

In the spring of 1930 a further test of hardiness was made. The weather failed to serve for chilling, and the plants, about 3 weeks of age, were placed in a refrigerator ice can at 8 a. m. At noon the temperature had reached 0° C., but the cultures were further chilled until 5.30 p. m. Plants of the Early Jersey Wakefield variety showed injury the next morning in the culture from beneath Vitaglass. With the Copenhagen variety there was no appreciable injury.

Of the cultures reared continuously under glass, by May 29 those under Vitaglass were about 2 inches lower than the others. The

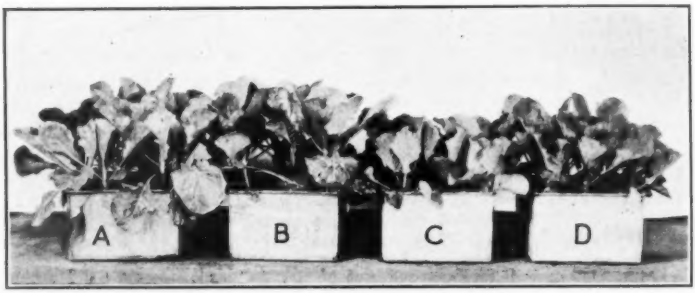

FIGURE 7.—All Seasons cabbage plants photographed June 6, 1929, at eight weeks of age: A and B were grown continuously under glass; C and D were exposed outdoors for a cold-hardiness test at three weeks of age; A and C were grown under Vitaglass and B and D under common glass

relative development shown in Figure 7, A and B, was recorded on June 6, when the culture under Vitaglass gave evidence of greater maturity by the shedding of basal leaves. When the plants were thinned on June 15 in the hope of promoting heading there was no difference in crude protein content of the tops, the values being 15.5 and 15.6 per cent of the dry matter. Despite an immediate application of KNO_3 and outdoor exposure until July 5, growth practically ceased and these cultures were abandoned.

RADISH (RAPHANUS SATIVUS)

On January 3, 1930, sowings of the Early Scarlet Globe and Grand Rapids Forcing varieties of radish were placed in beds of composted loam on the west-side bench of the greenhouse. These were thinned and tended in the customary manner. A census on February 13 showed practically no signs of root storage in about 165 plants of the Early Scarlet Globe variety under each type of glass. Of about 100 plants of the Grand Rapids variety under each glass 14 roots were filled under Vitaglass and 4 under common.

The areas in question were now planted to a type better adapted to production under glass. Early Scarlet Globe Superstandard was sown on February 15. When the plants were thinned on February 26 the area under Vitaglass contained small scattered plants, as compared with numerous vigorous ones under common glass. It should be observed that the Vitaglass area was subject to excess heat above the radiator intake. The removal on March 17 of certain plants which somewhat shaded the common-glass area promoted the proportion of filled roots. Harvesting occurred at intervals between March 17 and April 9 as the plants developed, the grand total yields of fresh tissue being as follows: 127 plants under common glass gave 708 gm. of tops and 557 gm. of roots; 146 plants under Vitaglass gave 552 gm. of tops and 606 gm. of roots. The production of tops was therefore better under common glass and the production of roots was better under Vitaglass. In another way the greater efficiency of root filling under Vitaglass is shown by the ratio of root to top yield, which was 1.10 under Vitaglass and 0.79 under common glass. The average root weight was nearly the same for the two treatments, or 4.16 gm. under Vitaglass and 4.41 under common glass. On two dates, March 17 and 29, the tissues were partly analyzed, with the results shown in Table 14.

TABLE 14.—*Effect of glass light screens upon the growth and composition of radishes*

Screen	Dry matter of top, Mar. 17	Dry matter of root, Mar. 17	Dry matter of top, Mar. 29	Dry matter of root, Mar. 29	Crude protein of root, Mar. 17	Crude protein of root, Mar. 29
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Vitaglass	6.9	4.9	9.1	5.0	18.9	15.1
Common glass	6.3	5.4	8.0	4.6	22.7	16.1

There is some consistency in relative dryness of top and lesser nitrogenous content of root under Vitaglass but more data would be required to justify conclusions.

LILIACEAE

ONION (ALLIUM CEPA)

Seeds of a white variety of onion were sown in flats early in April. After frequent clipping to induce bud development the crop of 18 plants per box was harvested on June 23, 1930. The roots were somewhat longer under Vitaglass. Table 15 gives the data obtained. The value for nitrogen in the bulbs under Vitaglass may well be questioned, but there was no material for verification. From these limited data it is only possible to infer that the fuller spectrum under Vitaglass was unfavorable to development of this genus, but less so to bulb than to top.

TABLE 15.—*Effect of glass light screens upon the yield and nitrogen content of onions grown under glass and harvested June 23, 1930*

Screen	Yield of green tops	Air-dry matter in tops	Nitrogen in tops	Weight of green bulbs	Air-dry matter in bulbs	Nitrogen in bulbs
	Grams	*Per cent*	*Per cent*	*Grams*	*Per cent*	*Per cent*
Vitaglass	18.5	8.7	1.99	21.6	13.0	0.78
Common glass	40.0	9.0	1.20	32.6	10.4	2.28

A hardiness test conducted in the refrigerator at about 0° C. for nine hours on May 5 resulted in serious injury to plants about 3.5 weeks old reared under common glass. However, these occupied a lower position in the freezing can than those from under Vitaglass and were probably subjected to a somewhat lower temperature.

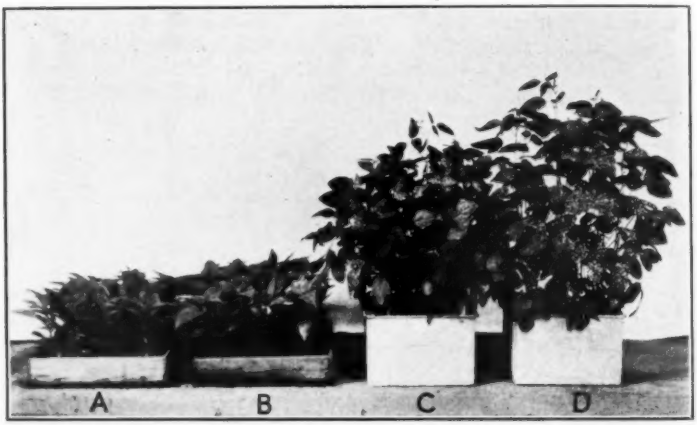

FIGURE 8.—Yellow coleus (A and B) and Wisconsin Black soybean (C and D) plants photographed June 6, at eight weeks of age, after continuous growth under glass. A and C were grown under Vitaglass and B and D under common glass

LEGUMINOSEAE

SOYBEAN (GLYCINE HISPIDA)

Seed from a commercial stock of the Wisconsin Black variety of soybean was planted in cubical boxes of loam on April 8, 1929. On May 1 the average height to stem tip under Vitaglass was 15 cm. and under common glass 12 cm. In thinning on this date 7 plants were taken and 10 left in each box. The green weights removed under the two glasses were essentially equal. By May 29 the plants in their natural posture were about 7 cm. taller under common glass than under Vitaglass. On June 1 many seed pods had formed under Vitaglass but only a few under common glass. The greater vegetative expansion under common glass on June 6 is apparent in Figure 8, C and D.

Most of the culture under Vitaglass were mature on July 10, three days earlier than under common glass. The plants were dried slowly

above a steam-heated oven to permit full translocation to the seeds. All brown pods and approximately the upper two-thirds of the plants were taken for obtaining data on yield and composition. The plumper seeds of each treatment averaged 152 mgm. in weight under Vitaglass and 135 mgm. under common glass, with total yields of 105 and 66 gm. under the respective treatments. The analytical results obtained are assembled in Table 16.

TABLE 16.—*Effect of glass light screens upon the percentage composition of soybeans grown under glass and harvested July 10–13*

Screen	Ether extract of seed	True protein of seed	Nonlipide, nonprotein nitrogen of seed	Ether extract of top	True protein of top	Nonlipide, nonprotein nitrogen of top	Hemicellulose of top	Crude fiber of top
Vitaglass	17.2	36.6	0.59	3.2	25.0	1.67	33	14
Common glass	18.1	34.2	.49	2.3	23.6	1.71	34	17

The only definitely significant variation in these data is the increased content of ether extract in the vines reared under Vitaglass. Further tests might enhance the importance of decline of crude fiber and increases of protein here observed.

Attempts to test performance of this species in the second generation were interfered with by insects. However, plants reared in late April and May, 1930, from seed of the original supply gave about 15 per cent greater height and leaf area under common glass than under Vitaglass, with a lesser difference in dry weights. There was no difference in the content of ether extract with change of glass, but the crude protein was 10 per cent greater under common glass than under Vitaglass.

COMPOSITAE

LETTUCE (LACTUCA SATIVA)

Lettuce was of particular interest by virtue of its leafy character and importance in the human diet. Golden Queen and Grand Rapids, head and leaf types, respectively, were sown in flats on September 28, 1929. At the second thinning on November 22, all cultures except Grand Rapids under Vitaglass retained 17 plants, and that had only 13. The average air-dry weight per plant at this time was greater under common glass for the leafy variety and under Vitaglass for the head-forming variety. As shown in Figure 9, the vegetative expansion of both varieties was greatest under Vitaglass on December 5. At this time light applications of KNO_3 and $Ca(H_2PO_4)_2$ were made in all flats. On December 16, following a prolonged period of low light intensity in cloudy weather, bacterial wilt was spreading rapidly in the Grand Rapids culture under Vitaglass. The margins of the leaves appeared to indicate slight osmotic injury from the salt applications. Consequently, the smaller pairs of flats under each type of glass were harvested, while the larger ones were further thinned, this selection involving 10 to 12 plants per flat. The dried tissues were subjected to chemical examination, with the results shown in Table 17. Despite the smaller size of the plants under Vitaglass, the constituents determined were present in remarkably uniform proportions under the two types of glass, with

the exception of a marked increase in lipide content of the head type under Vitaglass.

TABLE 17.—*Effect of glass light screens upon the yield and partial composition of lettuce harvested December 16, 1929, 79 days after sowing under glass*

Screen	Average air-dry weight per plant		Ether extract		Total non-lipide nitrogen, head type	True protein, leaf type	Non-lipide, non-protein nitrogen, leaf type
	Head type	Leaf type	Head type	Leaf type			
	Centi-grams	*Centi-grams*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Vitaglass	86	59	6.3	6.4	4.94	16.8	0.92
Common glass	90	75	5.0	6.3	5.07	16.7	.88

FLOWERING PLANTS

In view of favorable effects upon reproduction obtained under Vitaglass in some of the preceding tests, it appeared desirable to

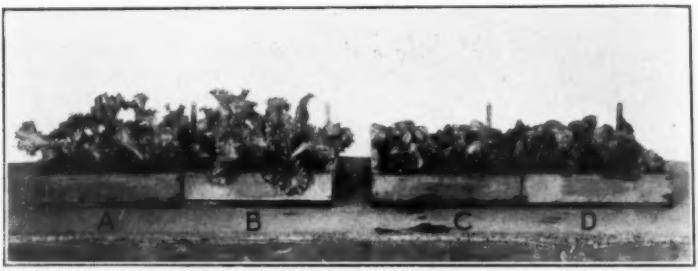

FIGURE 9.—Grand Rapids leaf (A and B) and Golden State Queen head (C and D) lettuce photographed December 5, 1929, at 10 weeks of age, after growth under glass: A and C were reared under common glass and B and D under Vitaglass

include in the present investigation a few species of floricultural value. On January 5, 1930, Chinese primrose (*Primula sinensis*) and ageratum (Hustonainum) were sown in beds of composted soil on the west bench of the greenhouse. By February 27 ageratum was more extensively vegetative under Vitaglass, while primula was about equally developed under the two forms of glass. On May 6 both species were in bloom and of much superior development under common glass. These responses were possibly complicated by excess of bottom heat from the intake of heating coils under the Vitaglass.

Potted cyclamen (*Cyclamen persicum*) and fairy primrose (*Primula malacoides*) in early bud placed under the special glass areas on November 25, 1929, developed blossoms two to three days earlier under Vitaglass than under common glass. Recently repotted cineraria (*Senecio cruentus* or *Cineraria hydrida*) and fairy primrose were subjected to the above-mentioned exposure beginning on December 10, and they also bloomed the earlier, January 2, 1930, under Vitaglass. These appeared equally developed under the two glasses on February 5. Due consideration must be given the possibility

that these effects could follow the greater heat transmission of Vitaglass.

Cuttings of begonia (*Begonia maculata* or *Argentia guttata*) set in sand were examined on February 3, 1930, after several weeks' exposure under the two types of glass. One-half the population of each flat (16 cuttings) showed no appreciable difference in root development. Continued exposure of the plants in potted form up to March 29 led to generally better development under Vitaglass, as shown in Figure 10. On May 10 the nitrogen content based upon samples from the tops of 10 plants gave under Vitaglass 2.36 per cent and under common glass 2.53 per cent.

In further tests with relatively small numbers of cuttings in both November and April neither glass gave decidedly superior rooting. Another test with young potted plants began early in February, and

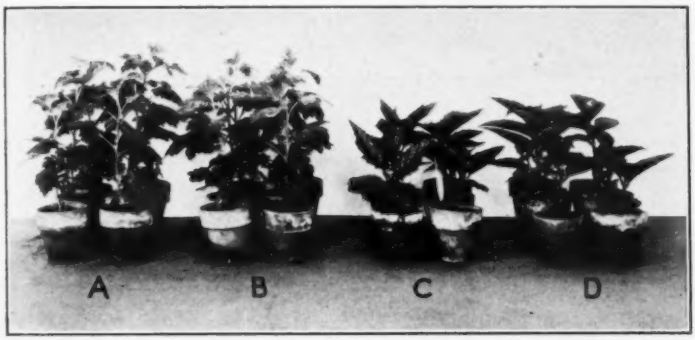

FIGURE 10.—Chrysanthemum (A and B) and begonia (C and D) plants reared from cuttings produced under glass covers and photographed April 1, 1930, eight weeks after transplanting; A and C were reared under common glass, B and D under Vitaglass

by the end of March the plants under common glass were more vegetative than the others, possessed more extensive root systems, and were slightly more advanced in blooming.

On April 10, 1929, cuttings of both red and yellow varieties of coleus (*Coleus blumei*) were prepared for exposure under the two types of glass. These were transplanted to flats of loam three weeks later. By May 20 the yellow variety was better developed and more highly colored under Vitaglass, while the red variety gave a less certain improvement under common glass. However, on June 6 the yellow variety was taller under common glass, as appears in Figure 8, A and B. When the tops were harvested a week later they provided the analytical data of Table 18. Both varieties show a higher content of ether extract under Vitaglass. While the red variety seems to have profited also in protein from this exposure, the yellow variety accumulated nonprotein nitrogen.

TABLE 18.—*Effect of glass light screens upon the percentage composition of coleus tops, June 13, after nine weeks' growth from cuttings*

Coleus variety	Screen	Percentage composition of tops		
		Ether extract	True protein	Nonlipide, nonprotein nitrogen
Red	Vitaglass	7.9	17.5	0.56
	Common glass	6.2	15.5	.48
Yellow	Vitaglass	5.9	13.6	1.49
	Common glass	5.2	15.5	.99

A second test with cuttings was set up November 20, 1929. On December 16, following a prolonged cloudy period, the yellow variety was more widely rooted over the base of the stem under Vitaglass and the vigor of root system of the red variety also appeared to be favored by this treatment. The plants were now potted, and on

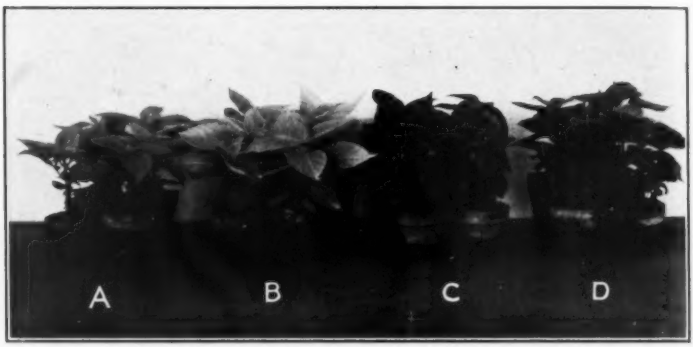

FIGURE 11.—Yellow (A and B) and red (C and D) varieties of coleus reared from cuttings produced under glass covers and photographed April 1, 1930, 15 weeks after transplanting. A and C were reared under common glass, B and D under Vitaglass

February 5 the vigor of tops was greater under Vitaglass, more particularly those of the red variety. On March 29 the leaves of the yellow variety under Vitaglass were bleaching and rolling, while the other variety had attained nearly equal development under the two types of glass. The root systems generally had extruded from the bottom of the pots under Vitaglass. In Figure 11 are displayed the larger, paler leaves of the yellow variety and the rather smaller and darker leaves of the red variety under Vitaglass.

A third lot of cuttings was made on April 15 from the plants just described. Inspection on May 7 of the small population showed 100 per cent rooting in all cases except the red variety derived under Vitaglass and tested under common glass. The tops of the red variety were also relatively poorly developed under common glass. Figure 12 shows the generally superior rooting of the yellow variety under Vitaglass.

Cuttings of a mixture of varieties of chrysanthemum (*Chrysanthemum hortorum*) were placed under glass October 23, 1929, and four weeks later they were examined. Here again the population was

too small (35 each) for conclusive results. In round numbers the percentage of total rooting was 100 under Vitaglass and 90 under common glass, with 95 and 80 per cent, respectively, of well-rooted

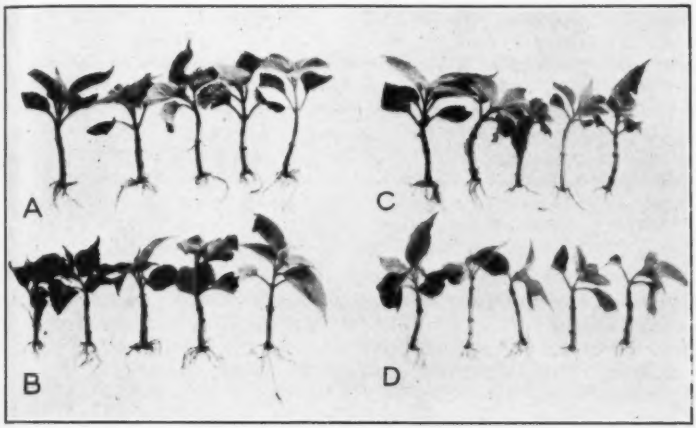

FIGURE 12.—Yellow coleus cuttings photographed May 7, 1930, after three weeks growth in sand; A and B under Vitaglass and C and D under common glass

cuttings. Under Vitaglass the better roots averaged 5.6 cm. maximum length as compared with 6.4 cm. under common glass.

This test was repeated, with examination of the cuttings on February 3, 1930. There was, as one might anticipate for solar radiation

FIGURE 13.—Chrysanthemums photographed May 7, 1930, after 13 weeks growth under glass, from cuttings. Plants in A were reared under common glass and in B under Vitaglass

of the winter season, no essential difference in development under the two glasses. The plants were potted at this time, and on March 29 they displayed the somewhat retarded development under Vitaglass which is apparent in Figure 10, A and B. As shown in Figure 13,

the plants under Vitaglass later advanced to flowering at the expense of vegetative enlargement, while those under common glass displayed only miniature terminal buds. The tops above the sixth internode were now taken as representative of the more chemically plastic tissues, and these furnished the data in Table 19.

TABLE 19.—*Effect of glass light screens upon the percentage composition of upper tops of chrysanthemum on May 8, after 18 weeks growth from cuttings*

Screen	Ether extract	True protein	Nonlipide, nonprotein nitrogen
Vitaglass	4.7	13.2	0.39
Common glass	4.9	14.5	.40

Despite the marked difference in development, an examination of the plant tissues under the two glasses showed no appreciable difference in composition. It is possible, of course, that samples from the region closer to the reproductive organs or even a general sample taken earlier would have disicosed compositional relations to blossoming performance.

There are likely to be varietal differences in behavior toward either increases in ultra-violet rays or the complex change resulting from replacement of common glass by Vitaglass. On October 15, 1930, a few potted plants were selected of the Gladys Pearson variety of chrysanthemum which were rather uniform in the early stages of bud development. A part of this selection was placed under each type of glass in the special greenhouse. Similar treatment was given a small yellow pompon variety. There are marked differences in the general character of these varieties, Gladys Pearson developing large buds and a few correspondingly large stems with rather pale leaves, while the yellow pompon produces many small buds on numerous stalks and dark-colored leaves. On November 1 several blossoms were opening on the Pearson variety while the buds were beginning to break on the pompon. Development was about equally advanced in the Pearson, which is evidence that there was no difference in heating effect under the two glasses. In the pompon bud opening was more advanced under Vitaglass. From this difference in behavior it would seem that the larger variety of chrysanthemum here tested was either too far advanced in bud development or otherwise unadapted to develop blossoming response under this particular exposure. The pompon variety, on the other hand, was apparently adapted and still amenable to stimulation by an increase in the shortest solar rays.

DISCUSSION

The relatively high transparency of Vitaglass to both ultra-violet and infra-red rays must be considered in attempts to explain plant responses of the kind here described. Although, as shown in Figure 2, excess heating of the atmosphere about the plants under this glass was largely compensated for by forced ventilation, infra-red rays were doubtless absorbed by the plant tissue to a greater extent under this exposure. The insertion of thermocouples should make evident any resultant heating effects within the plant, but there seems to exist

little information as to what other effects such rays may produce. Furthermore, it is clear that due consideration must be given the season of year concerned, as this affects the duration, intensity, and spectral distribution of solar radiation upon the plants. However, so far as its general range of transmission is concerned, Vitaglass surely affords radiation more closely resembling direct sunlight than that transmitted by common glass. This fact is of paramount importance in the present considerations.

Outstanding in the response of Marquis wheat under Vitaglass was its erect habit. (Fig. 3.) The effect was more prominent in this early summer period than in the late fall, when it resembled the results with winter wheats seen in Figure 4. This is not a temperature effect, for, as is well known, higher temperatures induce a flaccid, recumbent type of growth. It is at least remarkable that the difference in growth character acquired by early differences in the type of radiation received persisted after several weeks of common exposure to the outdoor environment. The results are suggestive of the continued functioning of directive substances, such as the hormones postulated in explanation of the dominance of apical over lateral buds. While the initial responses of the cultures to frost exposure indicated no lack of hardiness in the plants started under Vitaglass, the ultimate depression of grain yield bespeaks a greater temperature shock in this particular culture.

Apparently the growth responses of wheat were modified by both variety and season, for in the autumn Marquis was not appreciably larger under Vitaglass than under common glass, whereas it was in the spring. Two of the winter varieties were also larger under Vitaglass. Furthermore, Marquis wheat matured earlier under Vitaglass in the spring but later in the autumn. After the wheat had remained in a state of semidormancy for a prolonged winter period differences in performance and composition became obliterated, as though some unstable, directive factor had been diluted, masked, denatured, or even lost. In the harvest of spring-grown and summer-grown Marquis it appears that reproduction was markedly favored under Vitaglass. So far as the examination shows, the lipide content was considerably greater under Vitaglass than under common glass in young wheat plants, with lesser indications of parallel increases in one or another form of nitrogen compounds. It seems hardly probable that the increase of lipides was in this instance due to higher tissue temperature, for in earlier work (*26*) the percentage of this fraction was reduced by growth at higher temperatures. On the whole this species was disturbed more in form of growth and reproductive functions than in composition under the more extensive type of radiation.

As regards maize, injury by both frost and smoke indicate a looser form of tissue and less hardy type of growth under Vitaglass. Stimulation under exposure to this radiation is further indicated by increased size and earlier maturity. These characters were accompanied by increased proportions of lipides and protein in the tissue, which apparently carried over in part to the composition of the grain. In the infertile plants harvested in the winter these differences were not found.

The variety of tomato tested grew more rapidly and attained greater lipide content under Vitaglass. However, it was less hardy toward frost than when grown under common glass. In the tomato also

differences of composition disappear with the winter decline in solar radiation. The larger fruit under Vitaglass, together with increased content of acids and either lipides or the nitrogenous fractions, is suggestive of a higher plane of metabolic activity than under common glass. Perhaps this difference in performance after a quiescent winter period should be ascribed to immediate radiation differences, rather than to products previously formed by virtue thereof. In this connection it is well to recall the work of Murneek (*17*), who found marked metabolic monopoly of nitrogen compounds by the fruit of tomato. To what extent this demand is enhanced under Vitaglass remains problematical. The stimulation of early development noted here and with other species might be considered an index of greater nitrogen assimilation. As a matter of fact, one of the writers (*27*) found that ultra-violet rays promote absorption of nitrate by the young wheat plant, but the evidence for a difference between Vitaglass and common glass in this respect has been inconclusive in the present investigation.

Throughout the responses of such widely diverse species as potato, radish, and onion runs the common factor of increased proportion of storage tissue, in addition to marked depression of the vegetative phase, under Vitaglass. This form of response was not attended by appreciable changes in the chemical composition of the tissues. With due recognition of the fact that, as with enzymes and vitamins, minute quantities of directive substances might serve to shift the morphological expressions of metabolism, more discriminating analytical methods than were here employed would be required to determine this issue. In the case of the variety of cabbage tested, minor depressions of growth and cold hardiness were accompanied also by closely similar gross composition.

Particular interest attaches to the results obtained with the variety of soybean tested. Both the first and second generations were shorter under Vitaglass in the spring. A striking increase in the lipides of the straw in this case was accompanied by some suppression of crude fiber content and indications of higher proportions of protein. It is apparent that reproduction was especially favored under Vitaglass in this species, as expressed by both earliness of maturity and increased size and yield of seed. Thus the soybean becomes aligned with wheat and tomato in this survey as a plant that shows a simultaneous increase in lipide content and reproductive activity under Vitaglass. The question naturally arises as to whether the lipides are modified in character as well as amount, and what particular factor or factors introduced by the use of Vitaglass are responsible for the results. As to character, at least, it seems likely that the ultra-violet rays, because of their generally known activity, might function in an energizing capacity. On the other hand, the rapidity of development under higher temperatures is known to restrict desaturation of fats in the maturing seeds.

Undoubtedly some of the instances of earlier blooming in ornamental plants under Vitaglass could well be attributed to the slightly higher temperatures prevailing. So also might the rooting of cuttings, but it is noteworthy that the begonia was independent of such influence. On the other hand, coleus, and particularly the yellow variety, gave best rooting and subsequent development of the root system under Vitaglass. In connection with this behavior it displayed the in-

creased lipide content observed with other species under this type of radiation. The better rooting of chrysanthemum under Vitaglass could hardly be attributed to heat in the instance in which this exposure produced shorter but thicker roots. This effect resembles rather the relatively better development of storage roots and stems under Vitaglass. As a matter of fact, relatively high temperatures tend to exhaust carbohydrates by increased respiration, and the work of Reid (*20*) with certain species has shown that these constituents are primarily concerned in root development. In view of the limited population here dealt with, the preceding remarks should be considered as merely in the nature of tentative suggestions. The marked advancement in date of blossoming of chrysanthemum here observed without associated differences in grosser chemical composition certainly merits closer examination. If it is not found to be a temperature effect, search should be made for constituents which might possess selective absorption for the added ultra-violet radiations available under Vitaglass.

These results are at variance with the general statements of Sheard and his associates (*24*) regarding unspecified species of plants. They state that the height and size, together with the chlorophyll content of the fresh tissue, are greater under Vitaglass than under common glass. These relations of size do not hold generally in the writers' results, and rough comparisons of chlorophyll and carotinoid content on the dry basis indicate greater contents under common glass with wheat and coleus. However, as will appear presently, it would be quite possible for differences of plant variety and of various environmental factors to cause such variations in results.

In certain respects the present results conform to those obtained by Garner and Allard (*12*) in their investigations of photoperiodism. They concluded that the length of day "not only influences the quantity of photosynthetic material formed but also may determine the use which the plant can make of this material." The responses vary not only between species but between varieties of a given species. While the seed size of the early Mandarin variety of soybean was reduced when day length was 12 hours and less, the late Biloxi variety produced seeds of normal size when the day was shortened to 7 hours. The tuber: top ratio in the McCormick potato was greatest on a day of intermediate length, and the chrysanthemum flowered earlier on a relatively short day. Some of the short-day responses recorded by Garner and Allard were observed under Vitaglass in the present investigation.

It is difficult to conceive what common causal factors could operate in these two lines of inquiry. Inasmuch as the usual short day of Garner and Allard was limited to a midday period, increased proportions of ultra-violet should have been effective, as with the present tests under Vitaglass. However, they found that shading at midday also produced short-day effects. On account of the efficiency of extremely low intensities (3 to 5 foot-candles) of radiation from the Mazda lamp for increasing the length of day, Garner and Allard conclude that photoperiodic growth responses are divorced from photosynthetic activity. With Bacon (*14*), they found in certain species an accumulation of carbohydrates and a decrease of acidity in the upper stem in response to shortening of the day, and with initiation of flowering, but disclosed no general compositional relation. Like-

wise, Arthur and his associates (*6*) found no relation between the ratio of the more available carbohydrates to nitrogen contents and flowering activity of various species. However, it has been shown (*13*) that the response to photoperiod is relatively localized in the organs exposed and thus apparently independent of circulating agents. It thus appears possible that sufficiently localized analyses might disclose compositional relations to both duration and character of irradiation in cases where gross sampling and analyses have failed. The results of Denny (*9*) offer hope for significance of this procedure within feasible limits. Garner and Allard ascribe primary significance to a supposed decrease of protoplasmic hydration under the short day and assume the existence of relatively direct relations between radiation and the plant reactions finally expressed in growth responses. Bakhuyzen (*28*) similarly postulates a rôle of protoplasmic hydration in vegetative expansion, but includes a hypothetical hormone. It is conceivable that radiations transmitted either selectively or in greater proportions by Vitaglass than by common glass could activate certain receptive compounds of the plant to initiate the differences observed in the writers' test and at the same time function in the hydration hypothesis of Garner and Allard.

Despite the negative conclusions of Popp (*19*) and of Reinhold and Schulz (*21*), in certain respects their results resemble those obtained in the present investigation, and the writers are led to believe that they may have overlooked important limited responses in growth behavior and composition. Thus, the former recovered larger tomato plants and more fruit, together with slightly increased nitrogen content of the tissue, under a glass resembling Vitaglass in ultraviolet transmission. The other investigators here cited obtained increased yields and earlier ripening of tomatoes reared as seedlings under a glass resembling Vitaglass, together with increased root development of the radish.

Practical applications of the effects under Vitaglass here observed would seem to have distinct limitations as applied to plant production. However, for genetic studies the stimulation of seed production and shortening of the growth period should be advantageous. Moreover, the results of McCrea (*15*, *16*) indicate the practicability of enhancing medicinal potency of plants by transplantation to the field of seedlings reared under special glass. The results do not indicate commercial advantages in the production of such greenhouse crops as radish and tomato under Vitaglass. Further tests might demonstrate superior delicacy of leaf in such crops as lettuce, but such crops are readily provided out of season by modern transportation from field cropping in the United States. It could hardly be profitable to force the blooming of chrysanthemum and other ornamentals under special glass if suppression of size in both plants and blossoms is likely to follow. However, it must be recognized that several of the species here tested were not reared under optimal conditions of temperature. There appears, therefore, to be justification for further investigation of the practicability of introducing Vitaglass into greenhouse practice. In the case of certain cuttings and seedlings the present results indicate favorable growth rates by this treatment, and it would be desirable to compare the performance of these after transplanting them to the field.

As was indicated at the outset, the present investigation is hardly more than a limited survey. For conclusive results each species tested would require further examination. It is believed, however, that the more prominent results obtained justify further research. It will be desirable also to isolate the effects of infra-red from those of ultra-violet radiation. In further analyzing the plant response, particular attention should apparently be directed to the character of lipides in the leaf.

SUMMARY

Twelve species of plants, representative of four families of economic importance, have been tested as to growth performance and partial composition under Vitaglass. Similar cultures under common glass served as controls.

Some of these species gave only such growth responses as might be expected to result from increased temperatures due to the excess of infra-red radiation transmitted by Vitaglass.

Certain species which do not possess winter-hardening capacity were more subject to frost injury following early development under Vitaglass.

In various species seed production, root storage, or earliness of blooming were favored under the more extensive irradiation under Vitaglass.

In some instances an increased percentage of protein or of non-protein, nonlipide nitrogen was found in the tissues produced under Vitaglass, but the most consistent compositional response to this exposure was an increased percentage of lipides in the dry matter.

While mutual functioning of ultra-violet and infra-red radiations may be important in the use of Vitaglass, the desirability of separating these factors for comparison is indicated.

Some of the effects obtained under Vitaglass resemble those reported elsewhere in investigations of photoperiodism, but no causal factors common to the two cases are now apparent.

Possible applications of Vitaglass in commercial plant production are discussed, and suggestions are deduced relative to future experimentation.

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GRAY MOLD OF TOBACCO [1]

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INTRODUCTION

The prevalence of tobacco diseases in seed beds in North Carolina was investigated during the seasons of 1928, 1929, and 1930. Among the diseases found was one caused by a species of Botrytis to which little attention has previously been given and to which the name "gray mold" may appropriately be applied. This disease is characterized by a gray moldy coating which covers all affected parts. Apparently no one has studied the gray-mold disease of tobacco seedlings within the United States, although it has been investigated in certain other tobacco-growing regions. This report, therefore, embodies the knowledge of the present status of this disease, especially as regards its history, distribution, etiology, and the conditions governing its prevalence.

HISTORY, DISTRIBUTION, AND IMPORTANCE OF GRAY MOLD

Fragmentary notes constitute the only published accounts of Botrytis as being pathogenic to tobacco. The mention by Sorauer (*6*),[2] in 1886, of Botrytis on seedlings in seed beds in Germany appears to constitute the first account of this disease. De Toni (*12*), nine years later, noted a leaf spot of tobacco in Italy the cause of which he identified as *B. vulgaris* Fr.

In Peters's (*5*) compilation dealing with diseases and insect enemies of tobacco there is mentioned the occurrence in Java of *Botrytis cinerea* Pers. The author states that the stems of seedlings near the surface of the soil are involved in decay. Their roots and those of older plants become decayed, and large spots appear on the leaves. Ultimately the capsules are attacked, from which it is inferred that the fungus may be seed borne. He notes further that it may appear even on cured leaves.

The widespread occurrence of *Botrytis cinerea* on the fading flowers of various cultivated plants is a matter of common knowledge to plant pathologists. Pape (*4*) observed it on flowers of both *Nicotiana tabacum* and *N. rustica* in Germany. Diseased corollas that in falling chanced to lodge on leaves served as the inoculum for leaf spots. This observation has recently been confirmed by Böning (*3*).

Undoubtedly gray mold has existed in North Carolina for years, although no record of its occurrence as a seedling disease has been found. Neither is there any account of its presence in other tobacco-growing sections of the United States. Although it was noted in nearly every seed bed examined in 1928 and 1929, in a few cases only was it of serious consequence. Several seed beds were seen in which

[1] Received for publication Mar. 2, 1931; issued August, 1931.
[2] Reference is made by number (italic) to Literature Cited, p. 174.

gray mold was so abundant and so destructive that no plants were drawn from these beds for transplanting. In some cases the high mortality of plants set in the field was attributed by growers to the fact that the plants bore gray-mold lesions on the stems when they were pulled from the beds.

APPEARANCE OF THE DISEASE

Gray mold in North Carolina is essentially a disease of seedlings that are of sufficient size to be transplanted. It continues to develop throughout the remainder of the season on plants that remain in the beds after the close of the season for transplanting. Affected plants are, of course, usually discarded at the time of removal from the seed beds. If they are overlooked and chance to be transplanted in the field during a rainy period they may succumb; on the other hand, if transplanted in a loose, moist soil during a relatively dry period the larger proportion will completely recover.

The disease first appears on the lowermost leaves, during relatively dry weather, as large, brown, dry spots, bordered by a yellowish zone. During rainy periods wet-rot lesions are formed and the surface of invaded tissues is covered with a grayish coating of conidiophores and conidia. This coating constitutes a characteristic sign of the disease and is infallible as a criterion for diagnosis in the field. In favorable weather the blade is soon completely invaded and dries, though remaining attached, and the leaf collapses. Meanwhile the fungus has grown downward through the petiole into the stem. (Fig. 1.) The resulting stem lesions are dark brown to black and sunken. The size of these lesions appears to be governed by moisture conditions. In dry weather they remain small and the wound may completely heal. In wet weather, on the contrary, the lesion extends around the stem and the top is killed in consequence of this girdling. The blackened areas on stems may become several inches long and may be covered with a dense grayish coating similar to that on affected leaves. (Fig. 2.) If the leaves become detached and no conidiophores are present, the stem lesions may be confused with those produced by the sore-shin fungus, *Rhizoctonia solani* Kühn.

In seed beds abandoned because of the occurrence of this disease, from which no plants are pulled, all of the lower leaves generally become completely invaded and dry. Leaves higher on the stem bear large, dead patches. A similar condition obtains in beds in which the plants are still closely crowded after the season for transplanting is ended. This is especially noticeable in late June and July in beds that have not been plowed up. The etiolation of the older leaves, occasioned by the development of many plants in close proximity, appears to predispose the leaves to attack.

The appearance of affected tissues, as revealed by microscopic examination, shows that the mycelium of the pathogene is intercellular. The hyphae force their way between the cells as a result of their ability to produce a dissolution of the middle lamella. The cells behind the zone which is being invaded collapse and dry rather quickly. (Fig. 3.)

All leaf tissues are equally subject to attack, as shown in Figure 4, F. The extension of the mycelium between the cortical cells of the petiole

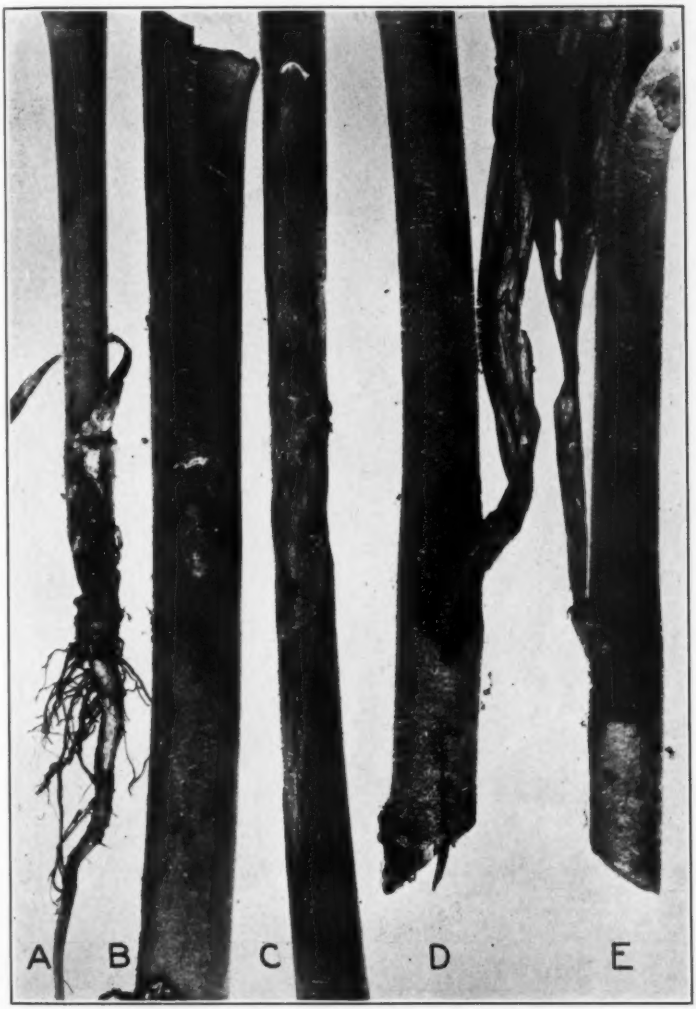

FIGURE 1.—Gray mold of tobacco. Plant A, girdled by fusion of several lesions originating at base of petioles. Plant B shows lesions produced at the nodes. Stem of C, girdled and a long lesion formed; portion distal to lesion dead. Two plants, D and E, show stem lesions originating from migration of Botrytis from leaf blade through petiole

is shown in Figure 5, A. Evidence of the splitting apart of cell membranes and of the pressure exerted by the hyphal tips as they force

apart the cells is presented in Figure 5, A and B. The vascular tissues of the petiole and stem are invaded in the same manner.

FIGURE 2.—Gray mold of tobacco; profuse growth of conidiophores covering surface of stems and leaves in damp weather. Note dense, white mycelial weft on surface of notch *a* cut in large stem at margin of lesion and coating of gray conidiophores on lesion. These growths resulted from placing the stems in a moist chamber for 24 hours

THE PATHOGENE

Gray mold of tobacco is caused by an organism that may be tentatively designated *Botrytis cinerea* Pers.[3] It is impossible to deter-

[3] Cultures and specimens were sent to Prof. H. H. Whetzel, Cornell University, Ithaca, N. Y., to whom grateful acknowledgment is made for this determination. Professor Whetzel from his study of Botrytis regards the tobacco gray-mold fungus as a member of the *cinerea* group.

mine from Persoon's description what fungus he had under observation, and therefore one can not be certain that the tobacco-seedling pathogene is specifically identical with the fungus described by him.

Some species of Botrytis are known only in the conidial stage, while others possess in addition an ascogenous and a sclerotial stage. The tobacco pathogene forms both conidia and sclerotia in culture. The hyphae are coarse and vacuolate, both in lesions and in culture. (Fig. 4, D.) Each conidiophore consists of a stout, erect branch with a few lateral branches on which botryose clusters of conidia are borne.

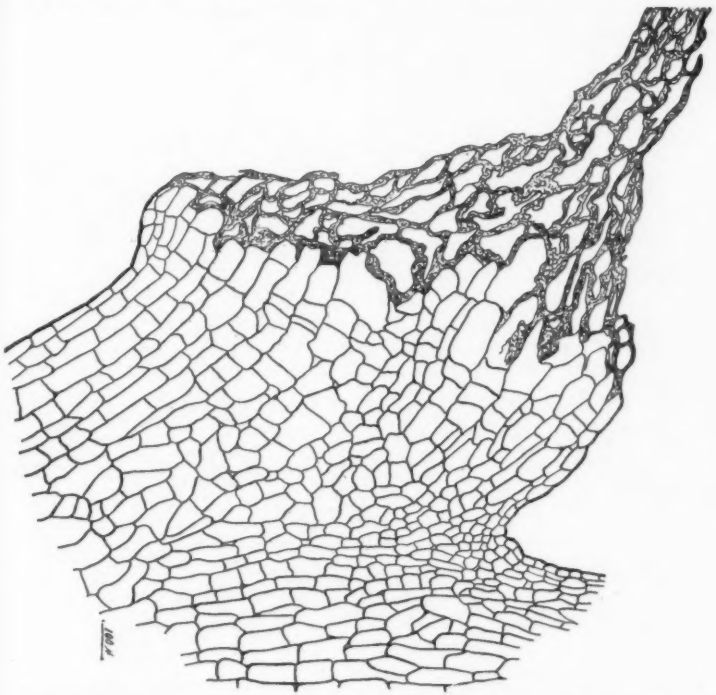

FIGURE 3.—Section of the petiole of a tobacco leaf in the region of its attachment to the stem and at one side of the center, showing the extension of a gray-mold lesion from the leaf blade through the petiole toward the stem. Those cortical tissues that have been invaded are in different stages of collapse

(Fig. 4, A.) The conidia are oval and are attached at the bluntly tapered end. (Fig. 4, B.)

A vigorous growth of grayish mycelium occurs on any of the ordinary culture media. Holdfasts arise at points where the mycelium comes in contact with the walls of the culture flask or test tube. (Fig. 4, E.)

The inoculation of tobacco seedlings with watery suspensions of conidia from pure culture resulted in infection only when a relatively high humidity was maintained. When inoculated plants were covered to conserve moisture they became infected, while those that

remained uncovered and from which the drops of suspension rather quickly evaporated remained free from infection. When *Botrytis cinerea*, isolated from decaying strawberries, was used as an inoculum, infections on tobacco resulted under the same conditions of inoculation. The inoculation experiments demonstrate that *B. cinerea* is pathogenic to tobacco only when conditions are made to approximate those in the seed bed. These conditions include the maintenance of films of moisture on the leaves for extended periods and the presence of leaves that are more or less weakened by age or by shading. This dependence on environmental factors accords with the writer's observations on diseases caused by Botrytis on lettuce, dewberries, and strawberries.

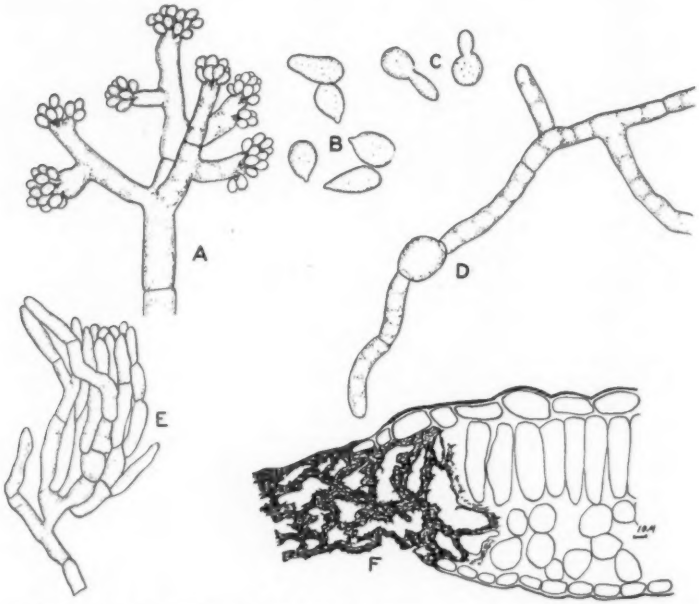

FIGURE 4.—A, Conidiophore of *Botrytis cinerea;* B, conidia; C, initiation of conidial germination; D, hyphal formation; E, a young holdfast; F, margin of a leaf lesion; all invaded portions are completely collapsed

The observations of Peters (*5*) on the occurrence of *Botrytis cinerea* as a parasite on tobacco seedlings and as a saprophyte on cured leaves and those of Pape (*4*) and of Böning (*3*) on its presence as a semiparasite on fading flowers indicate the possibility of the same species being concerned in the stem rot, poleburn, or house burn of tobacco investigated by Sturgis (*9*, *10*) in Connecticut, Virginia, and Kentucky. Sturgis noted this decay on tobacco cut during warm, foggy weather and hung in curing barns, and identified the primary cause as *Botrytis longibrachiata* Oud.

Under the name "Dachbrand" or "Rippenfäule" Behrens (*2*) studied a decay of tobacco in curing barns in Germany, presumably identical with the stem rot described by Sturgis (*9*). In his opinion

the causal organism is *Botrytis cinerea*, of which *B. longibrachiata* is merely a strain or form. However, Sturgis's (*11*) further report of stem-rot studies maintains that *B. longibrachiata* and *B. cinerea* are easily separable because of differences in habit of growth, in color, and in size of conidia.

Whether the same species of Botrytis is responsible for the seedling disease in North Carolina and abroad, and whether it can also attack fading flowers and leaves that are curing, and, in addition, can produce a decay of cured leaves, can not be determined at this time. However, when the specific limits of *B. cinerea* are determined, the

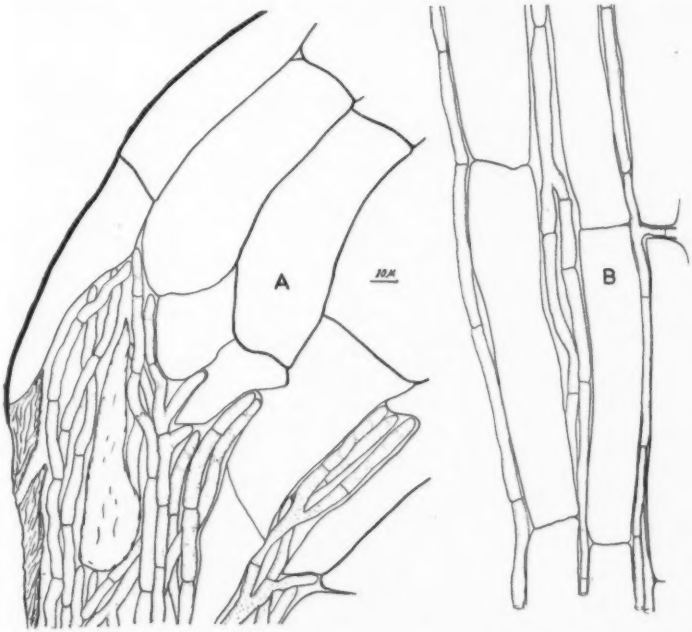

FIGURE 5.—A, Longitudinal section of a petiole showing the margin of a lesion. The hyphae of *Botrytis cinerea* are forcing apart the cells of the cortex after the dissolution of the middle lamella. B, Intercellular hyphae of Botrytis in the cortex of a stem lesion

possible identity of *B. vulgaris*, *B. longibrachiata*, and *B. cinerea* can be established.

INFLUENCE OF CLIMATIC CONDITION

The gray-mold fungus was found in nearly all of approximately 250 seed beds observed during the spring of 1928 and of 1929. In contrast with this condition, it was noted in only two of the 257 seed beds examined in 1930. An attempt was therefore made to correlate moisture and temperature conditions with the general prevalence of gray mold of tobacco during these two successive years and by this same procedure to account in large part for its almost entire absence during 1930.

It should be recalled in this connection that diseases caused by Botrytis are known to be favored by excessive rainfall and high relative humidity. Specific attention has been called to this fact by Stevens and Wilcox (*8*) in the case of a rot of strawberry fruits, both in the field and during transit. Moreover, studies on the temperature relations of the gray-mold fungus (*7*) show that it grows best at 25° C. (77° F.), that its minimum is near 0° C. (32° F.), and that it is rather sharply impeded at 30° C. (86° F.).

The meteorological data [4] for the period during which the tobacco seedlings were developing in the seed beds are assembled in Table 1.

TABLE 1.—*Precipitation during winter months of seasons 1927–28, 1928–29, and 1929–30, and precipitation and temperature during spring months of 1928, 1929, and 1930, at Oxford, N. C.*

Month and year	Precipitation	Mean temperature		Days with indicated temperature		
		Maximum	Minimum	Maximum of 68° F. to 85° F.	86° F. or above	34° F. or below
	Inches	*° F.*	*° F.*	*Number*	*Number*	*Number*
December, 1927	4.07					
January, 1928	1.21					
February, 1928	3.03					
Total, winter months	8.31					
March, 1928	2.95	62	38	10	0	1
April, 1928	6.93	68	43	17	0	4
May, 1928	2.50	76	53	24	4	0
Total, spring months	12.38			51	4	5
Total, 6 months	20.69					
December, 1928	.93					
January, 1929	1.48					
February, 1929	7.24					
Total, winter months	9.65					
March, 1929	5.38	66	43	12	2	5
April, 1929	4.30	74	48	20	4	1
May, 1929	4.54	79	54	26	5	0
Total, spring months	14.22			58	11	6
Total, 6 months	23.87					
December, 1929	1.97					
January, 1930	2.27					
February, 1930	1.70					
Total, winter months	5.94					
March, 1930	1.57	60	35	7	0	15
April, 1930	2.32	71	44	15	3	5
May, 1930	3.70	81	56	22	7	0
Total, spring months	7.59			44	10	20
Total, 6 months	13.53					

The influence of weather on this gray-mold disease may be better understood by considering the rainfall for the three preceding winter months in connection with the data assembled in Table 1. The total precipitation from December 1, 1927, to February 29, 1928, was 8.31 inches, which, with 12.38 inches for the spring months,

[4] Thanks are extended L. A. Denson, meteorologist, United States Weather Bureau, Raleigh, N. C., for making these data available.

makes a total of 20.69 inches for the 6-month period from December 1, 1927, to May 31, 1928. The total rainfall for the three winter months, December 1, 1928, to February 28, 1929, was 9.65 inches; for the three spring months, March 1, 1929, to May 31, 1929, 14.22 inches, making a total precipitation of 23.87 inches for the entire 6-month period. The total rainfall from December 1, 1929, to February 28, 1930, was 5.94 inches; for the three succeeding spring months, 7.59 inches, making a total for the half year of 13.53 inches.

The weather records for Oxford, N. C., do not cover a period sufficiently long to establish a normal, but the average normal for Henderson, N. C., and Durham, N. C., two near-by stations, is approximately 21.50 inches for the corresponding half year. The 1928 season, therefore, shows an approximate total deficiency of 0.81 inch; the 1929 season, an excess of 2.37 inches; and the 1930 season, a deficiency of 7.97 inches.

It may be noted from the tabulation that the precipitation for the three spring months of 1928, 1929, and 1930 was 12.38 inches, 14.22 inches, and 7.59 inches, respectively. The average normal for this period is 11.33 inches, making a departure from this normal of +1.05 inches, +2.89 inches, and −3.74 inches, respectively, for the three successive seasons. The season of 1930 was, in fact, so dry that the seedlings would have succumbed if growers had not watered the seed beds.

During the 92-day period while the seedlings were in the seed beds there were 51, 58, and 44 days in 1928, 1929, and 1930, respectively, during which the maximum temperature ranged from 68° to 85° F. for a portion of the day. This range may be regarded with fairness as optimum for the growth of the gray-mold organism. Unfavorable temperatures of 86° or above prevailed for 4, 11, and 10 days, and of 34° or below for 5, 6, and 20 days in 1928, 1929, and 1930, respectively, making a total of 9, 17, and 30 days on which unfavorable temperatures for the growth of Botrytis prevailed for a portion of the day. There were, therefore, approximately twice as many periods in 1930 as in 1929 during which growth was hindered by unfavorable temperature and more than three times as many in 1930 as in 1928.

Peters (*5*) points out that gray mold in Java appears during moist weather. Behrens (*1*) noted that it attacks tobacco seedlings in Germany when excessive humidity is maintained in the beds. The present observations on the influence of climatic conditions on gray mold, therefore, accord with previous observations on diseases induced by Botrytis on tobacco and other plants.

CONTROL

No definite experiments have been conducted to determine means of prevention and control of gray mold. However, observations show that the disease is not equally destructive in all seed beds. Those situated on low-lying ground which is poorly drained and which in consequence remains wet during seasons of normal rainfall may be seriously damaged. This condition is further aggravated if proper air drainage is prevented by a dense growth of woods surrounding the seed beds. In fact, all severe infestations that have been seen occurred in situations of this type. If the beds are located on porous soil on well-drained slopes in situations where the movement of air

currents is unimpeded, there is little probability of material damage by gray mold. The proper selection of a site for the seed beds, therefore, appears to afford a satisfactory means of prevention.

SUMMARY

Gray mold of tobacco in North Carolina is caused by a fungus tentatively identified as *Botrytis cinerea*. This disease is undoubtedly identical with diseases attributed to this and other species of Botrytis in Germany, Italy, and Java. There is a probability that the same organism may be associated with "stem rot" of tobacco in curing barns in Connecticut, Virginia, and Kentucky.

Gray mold is mainly a disease of seedlings in the seed bed. It first appears on the lower leaves that are predisposed to attack because they are past maturity and are etiolated. The pathogene passes from the leaf into the stem, where dark, sunken, necrotic lesions are formed. In extreme cases the stems are girdled and the plants succumb while in the seed bed or after being set in the field. Affected parts are covered with a grayish coating of conidiophores and conidia.

The epiphytotics of gray mold in 1928 and 1929 are correlated with abundant rainfall and a large proportion of days when the temperature was optimum for the growth of the pathogene. The scarcity of this disease in 1930 is correlated with the dry season, the relatively small number of days with optimum temperatures for the growth of Botrytis, and the relatively large number of days with temperatures that impeded the growth of the pathogene.

Seed beds located on well-drained slopes with good air drainage are least liable to be attacked by gray mold.

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CONTROL EQUIPMENT FOR THE STUDY OF HARDINESS IN CROP PLANTS [1]

By GEORGE L. PELTIER

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INTRODUCTION

The ordinary method of determining hardiness in crop plants has been to plant diverse sorts in many regions, and after a thorough test over a period of years, to propagate and distribute those kinds that survived the prevailing environmental conditions and were productive locally. This method of testing for hardiness is necessarily slow and frequently inaccurate, owing to the great variability from year to year of the many and complex environmental factors involved.

In consequence of the continual search for hardy as well as productive crop plants, through numerous collections, selections, and hybridizations, the number of species, varieties, and strains have increased so rapidly that the old methods are inadequate for testing the influx of material. Within recent years there has developed a tendency to employ control equipment for the rapid testing of hardiness in crop plants. Winter-hardiness tests of a number of crop plants with control equipment have been reported from the Kansas and Minnesota Agricultural Experiment Stations and from the laboratories of Åkerman in Sweden and Maximov in Russia. In 1927 control freezing equipment was installed in the plant pathology greenhouses at the Nebraska Agricultural Experiment Station for use in the study primarily of hardiness in alfalfa. A brief description of this installation is presented in order to acquaint workers in this field with the type of equipment employed in hardiness tests with alfalfa, which will be reported from time to time from this station.

THE HARDENING ROOM

The control equipment consists essentially of three rooms, one for hardening, one for freezing, and one for storage. The hardening room (fig. 1) consists of a greenhouse (12 by 25 feet) with double glass, having 2 inches of dead air space on the roof, sides, and partitions. A low and uniform temperature is maintained in this greenhouse by the principle described by Peltier and Goss.[2] In brief, it consists in drawing air from the greenhouse over brine cooling coils and discharging the cooled air back into the greenhouse. In order to maintain an air temperature of slightly above freezing in the greenhouse during relatively warm weather, a rather large cooling system is necessary.

The refrigerating system includes an ammonia compressor (capacity 7 tons), a brine tank (20 by 4 by 4 feet), ammonia cooling coils

[1] Received for publication Feb. 9, 1931; issued August, 1931. Paper No. 104 of the Journal Series of the Nebraska Agricultural Experiment Station.

[2] PELTIER, G. L., and GOSS, R. W. CONTROL EQUIPMENT FOR THE STUDY OF THE RELATION OF ENVIRONMENT TO DISEASE. Nebr. Agr. Exp. Sta. Research Bul. 28, 16 p., illus. 1924.

(1,600 linear feet), a centrifugal pump for circulating the brine through coils (2,700 linear feet) in a bunker (22 by 5 by 6 feet), and a blower (capacity 6,000 cubic feet per minute) for circulating the cooled air, together with various automatic control devices. The compressor, a self-contained unit, is located in the basement of the head house, while the well-insulated brine tank and bunker (figs. 2 and 3) are located directly under the greenhouse. The brine tank has a capacity sufficiently large to build up a sizable cold reserve. A temperature of about −30° C. is maintained in the brine tank in order to take care of the peak load during warm, sunny days. The compressor is connected with an immersion Mercoid thermostat which automatically regulates the brine temperature.

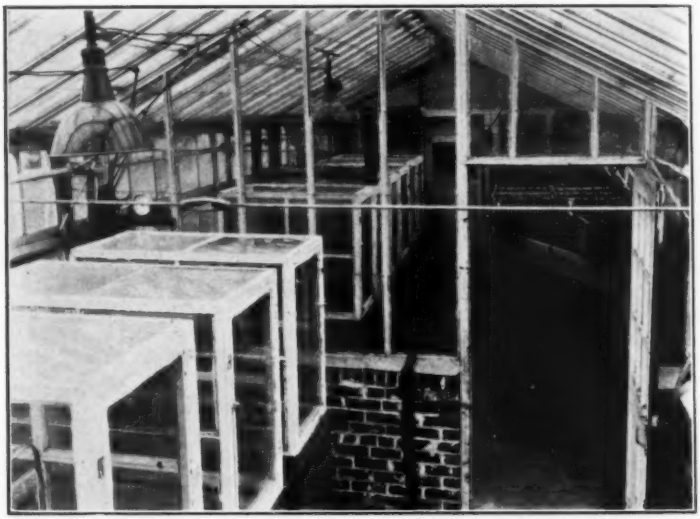

FIGURE 1.—General view of hardening room in the greenhouse showing bench for hardening plants, low-temperature cases, and, in the background, top of air duct. A temperature of slightly above 0° C. is maintained in this room from October through the month of May

The bunker coils are built in three units made up into sections 3 pipes wide and 14 high. These units are separated by baffles so that the incoming air must pass over each unit in succession before it is discharged into the greenhouse. The coil units can also be operated separately and can be shut off either when not essential or when they are in need of defrosting. By means of a Mercoid thermostat in the greenhouse the brine pump and the blower are automatically regulated.

The air is taken from one end of the greenhouse over the brine coils in the bunker and discharged by the blower through a specially constructed air duct at the top of the greenhouse. The air duct (fig. 4) is so constructed that the cooled air is evenly distributed to all parts of the greenhouse. A uniform temperature of near 2° C. is usually maintained in the greenhouse with a variation of ±2°, which is the limit of accuracy of the Mercoid thermostat. The hardening

room is used during the months of October through May and is employed primarily to harden plants prior to freezing. In most of the experiments the plants are held in this greenhouse for a period of two weeks.

For more accurate control of low temperatures the tanks and cases described by Peltier and Goss [3] are used. These are placed in the hardening room (fig. 1) and temperatures maintained in them within a range of ±1° C. by heating back with heating elements controlled by electric thermostats.

The pony relays, storage batteries, and mercury thermostats used by Peltier and Goss [3] to maintain temperatures in the tanks have

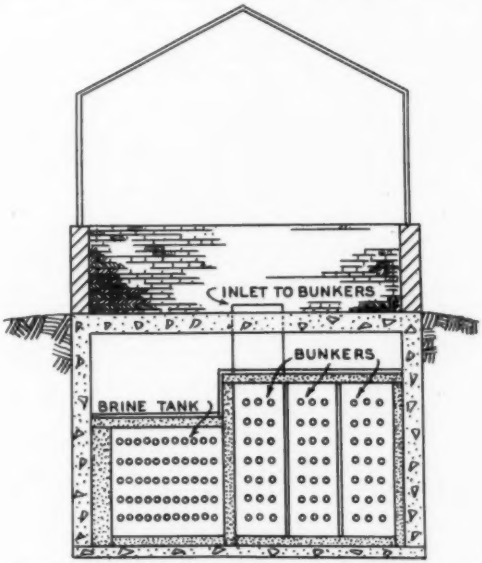

FIGURE 2.—Section of the hardening room showing construction and arrangement of brine tank and bunkers

been replaced with immersion Mercoid thermostats which can be connected directly with the electric current. Likewise, automatic switches have replaced the pony relays and batteries in the temperature cases. In both instances a more accurate and dependable control is obtained, as the old type of equipment was a continual source of trouble unless it was closely checked at least three times a day.

THE FREEZING ROOM

The sharp-freezer room (8 by 7 by 8 feet) is an ordinary, well-insulated compartment, located in the head house directly opposite the hardening room. The ammonia coils (208 linear feet) are located near the ceiling so that the cold air settles down on the plants under test, which are placed on stands about 3 feet from the floor. A

[3] PELTIER, G. L., and GOSS, R. W. Op. cit

slowly revolving ceiling-type fan, with two narrow, long blades, assists in distributing the cold air evenly throughout the room. The temperature of the room is regulated by a Mercoid thermostat (range ±2° C.) connected with an automatic valve between the

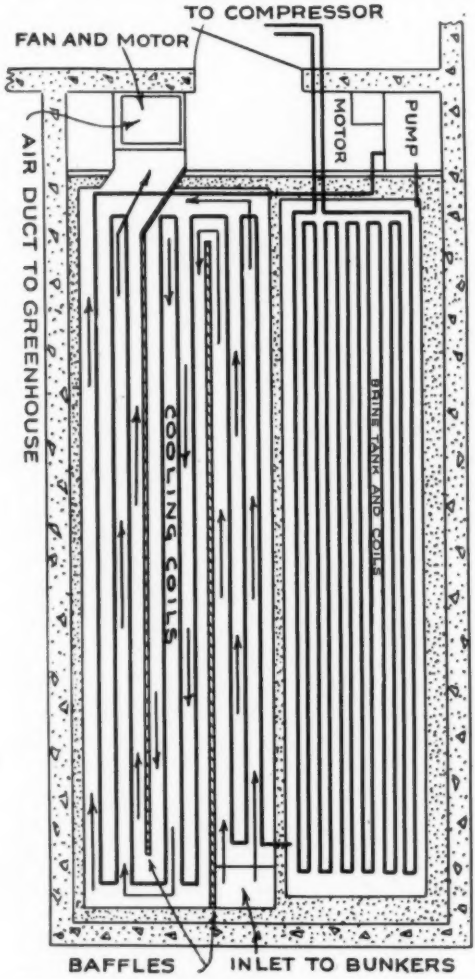

FIGURE 3.—Details of arrangement of refrigerating system beneath the hardening room

ammonia coils and a small compressor (capacity 1½ tons). Temperatures as low as −30° can be easily maintained for indefinite periods.

In order to avoid wide fluctuations in the temperature of the freezer entrance is made through an outer room of the same size

and construction but without ammonia coils. By means of a 16-point Leeds and Northrup resistance thermometer recorder an exact check is maintained of the room, plant, and soil temperatures of the materials under test. For convenience and checking an L-shaped thermometer is inserted through the insulated wall and the temperature of the room ascertained by the thermometer reading outside the freezing room.

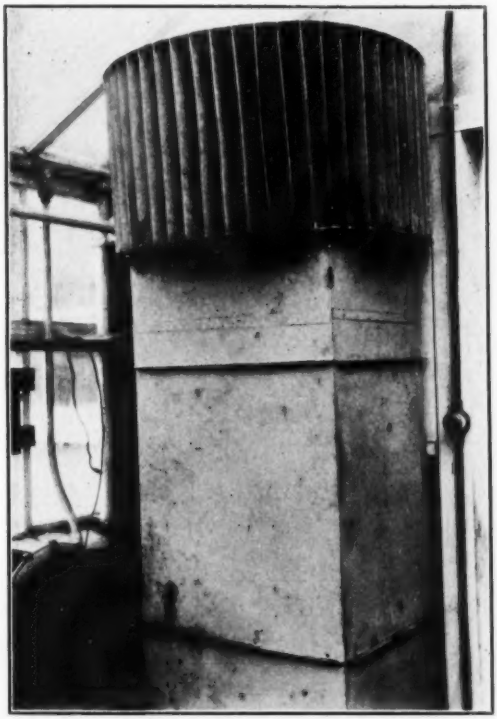

FIGURE 4.—Air duct for distributing the cooled air uniformly throughout the hardening room

THE COLD-STORAGE ROOM

The storage room (24 by 8 by 8 feet) is located in the basement of the head house directly under the freezing room, and is of the same construction. A temperature of about 2° C. is maintained in this room by means of a Mercoid thermostat (range ±2°) connected through an automatic ammonia valve to the same compressor that furnishes refrigeration to the freezing room. By means of these automatic valves, it is possible to maintain even temperatures in two compartments at wide differentials. Thus, by the use of only one compressor, a temperature of near 2° is held in the cold-storage room, while temperatures as low as −30° are possible in the freezing room.

The cold-storage room is used for a number of purposes, and the low-temperature incubators described by Peltier and Goss [5] are held in this room. In the hardiness studies the room is employed for hardening tests and storage of plant materials when light is not essential.

SUPPLEMENTARY EQUIPMENT

Supplementing the low-temperature equipment is a head house 80 by 20 feet fully equipped to handle efficiently all experimental work of the department, together with two green-houses (28 by 100 feet), each divided into three sections, and one small greenhouse (12 by 100) which is so arranged that it can be used for all studies dealing with the relation of environment to disease. All in all, the practicability and efficiency of the facilities and equipment have proven satisfactory during the three years in which they have been in operation, although improvements are being added from time to time.

[5] PELTIER, G. L., and GOSS, R. W. Op. cit.

EFFECT OF CHLORATES UPON THE CATALASE ACTIVITY OF THE ROOTS OF BINDWEED[1]

By J. R. NELLER[2]

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INTRODUCTION

Although it is known that chlorates cause a marked poisoning of plants when sprayed or dusted upon them, the nature of this poisoning is still largely undetermined. Since sodium chlorate, for example, is much more toxic than sodium chloride it is apparent that the chlorate ion is the toxic part of the salt. In an endeavor to learn more about the toxicity of this ion, the catalase studies reported in this paper were made. The bindweed (*Convolvulus arvensis*), also known as wild morning-glory, was selected for this work because its root system extends several feet in depth and thus permits a study of the progressive action of the chlorate upon portions of the plant not directly touched by the spray. A study of the catalase of the roots of unsprayed plants was also of interest since there appeared to be no mention of the subject in the literature on catalase activity.

At the same time it was thought that catalase-activity determinations might aid in the general problem of weed eradication experiments with chlorates already under way, as reported by Schafer, Lee, and Neller (*9*).[3] It seemed possible that the condition of the roots might thus be determined in a more accurately quantitative way than is obtainable by a visual examination.

HISTORICAL REVIEW

There appears to be a definite relation between the growth or nutritional condition of plants and their catalase activity. Thus Auchter (*2*), Heinicke (*6*), and Knott (*7*) have found that high catalase activity is associated with high vigor in plants. Davis (*4*) found that the catalase content of dead seeds was not retained so well as that of seeds capable of germination. Although Ezell and Crist (*5*) report that rapid growth was associated with low catalase activity, their findings are not necessarily in conflict with those of the authors mentioned above for the reason that considerable catalase may be used up in a rapidly growing plant. During the rest period after a period of rapid growth catalase activity might be found to be higher than in a plant that had shown less vigor and growth.

Under abnormal or pathological conditions, as in the physiological breakdown of apples,[4] it has been shown that catalase activity may be higher for a time but that eventually it becomes lower than in normal tissue. Overholser (*8*) recently reported that the freezing of

[1] Received for publication Nov. 3, 1930; issued August, 1931. Scientific Paper No. 172, College of Agriculture and Experiment Station, State College of Washington.

[2] It is a pleasure to acknowledge the helpful cooperation of Profs. E. G. Schafer and A. L. Hafenricter, of the Department of Agronomy, in connection with the agronomic phases of this investigation.

[3] Reference is made by number (italic) to Literature Cited, p. 189.

[4] NELLER, J. R. RELATION OF CATALASE ACTIVITY TO PHYSIOLOGICAL BREAKDOWN IN JONATHAN APPLES. Wash. Agr. Expt. Sta. Unpublished data.

pear tissue resulted in greatly reduced catalase activity. Carrick (*3*) has reported similar data for apple tissue. These findings lead to the conclusion that an impairment of the vigor of a plant is reflected in a reduction of its catalase activity.

PROCEDURE

During the 1929 growing season root samples were obtained from field plots that had been laid out in areas so infested with the bindweed as to exclude other vegetation. Some of the samples of 1929 and all of those of 1930 were taken from plots from which wheat had been harvested and the only green growth remaining was bindweed. To obtain the samples, the soil was removed in foot sections with an 8-inch auger, several borings per plot being made to obtain a composite sample. The samples were immediately taken to the laboratory and all the bindweed roots that could be found were picked out and their catalase activity measured. Preliminary experiments had shown that the activity of the roots increased after a few hours even when they were kept in a refrigerator. Samples were prepared by grinding 2.5 gm. of fresh roots in a porcelain mortar with an equal weight of calcium carbonate, a quantity sufficient to provide for an excess throughout the determination. Enough acid-treated washed sand and distilled water were added to grind the mixture to a smooth paste. This was made up to 50 c. c., and 20 c. c. portions were pipetted into the reaction bottles. Ten cubic centimeters of 3 per cent hydrogen peroxide were used for each determination. This contained 100 c. c. of available oxygen, and the amount of sample was controlled so that not more than 70 c. c. were liberated. The hydrogen peroxide was neutral to methyl orange and liberated no gas when used alone with the calcium carbonate.

The method employed for the catalase determinations was similar to that reported by Appleman (*1*) except that the apparatus was somewhat different. A water bath containing a wide-mouthed reaction bottle of 60 c. c. capacity was used. A 3-hole rubber stopper fitted into this bottle, one hole being for the stirring mechanism, one for the ingoing hydrogen peroxide, and one for the outgoing oxygen. The glass stirrer was driven by a motor equipped with a variable-speed reducer and was lubricated in its contact with the rubber stopper by a mixture of powdered graphite and glycerin. The hole in the stopper was cut larger than the glass stirring rod to allow a piece of rubber tubing to cover the rod where it passed through the stopper. It was found that a gas-tight bearing was thus obtained that could easily be kept lubricated with the graphite-glycerin mixture. A hydrogen peroxide chamber, consisting of the bulb part of a 10-c. c. pipette, was built into the system by using two U-shaped glass T's. This enabled the operator to allow the hydrogen peroxide to mix with the catalase suspension after the entire system had been closed and the stirring started without causing a change in the initial gas volume of the apparatus. During the course of the reaction the leveling bulb was lowered occasionally so as to maintain an approximate atmospheric pressure within the system. Readings were taken from the gas burette at 1-minute intervals with the use of a stop watch. All determinations were made at a temperature of 20° C. At first a record of air temperatures and barometric pressures was kept, but this was not continued as it was found that the greatest correction of

the gas volumes thus obtained was considerably less than the variation between samples.

RESULTS

In August, 1928, sodium chlorate at the rate of 484 pounds per acre was applied as a spray to bindweed in a group of field plots, and in March, 1929, samples of soil were taken from the first foot section of two of the plots. The results, which are shown in Table 1, indicate

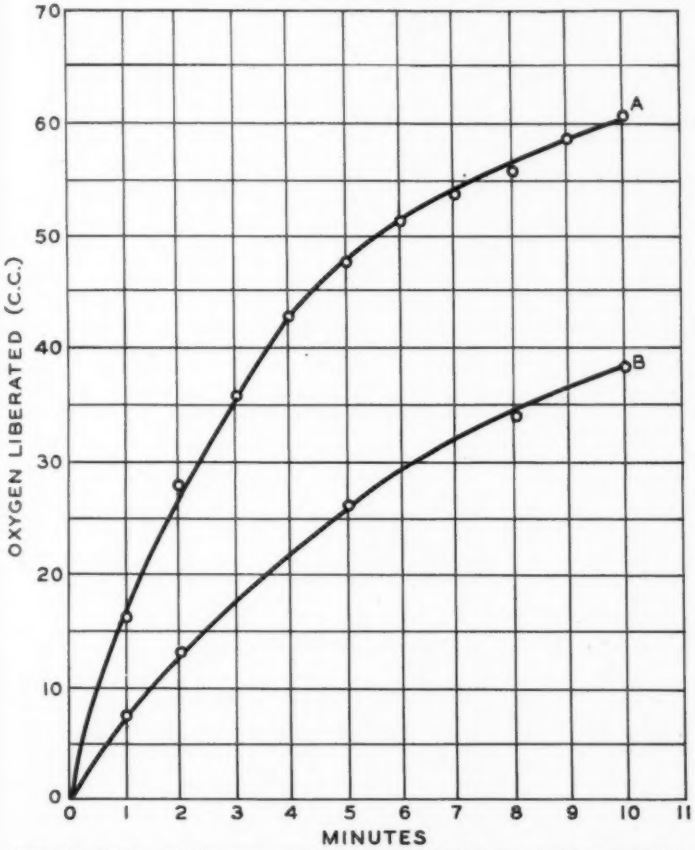

FIGURE 1.—Catalase activity of normal (A) bindweed roots and of roots from bindweed which had been sprayed with sodium chlorate (B); samples taken from the surface foot of soil in March, 1929

that the chlorate had a distinctly retarding effect on the catalase activity of the bindweed. The amounts of oxygen liberated at the end of 1, 2, 5, 8, and 10 minutes from untreated roots were 122, 109, 81, 63, and 59 per cent greater, respectively, than those from treated roots. (Fig. 1.) Since these results indicated that the maximum differences in catalase activity occurred during the early stages of the

reaction, subsequent comparisons were based upon catalase activity measurements of from 2 to 6 minutes.

TABLE 1.—*Effect of sodium chlorate upon catalase activity of bindweed roots taken from the surface foot of soil in March, 1929*

Treatment of plots	Duration of test	Oxygen liberated		
		Aliquot 1	Aliquot 2	Average
	Minutes	*C. c.*	*C. c.*	*C. c.*
None	1	16.8	16.9	16.9
	2	28.7	28.8	28.8
	3	36.5	36.9	36.7
	4	42.8	43.6	43.2
	5	47.7	48.4	48.1
	6	51.4	52.4	51.9
	7	52.7	55.3	54.0
	8	55.7	57.6	56.7
	9	58.6	60.0	59.3
	10	60.2	61.8	61.0
Sodium chlorate [a]	1	8.3	6.9	7.6
	2	15.0	12.5	13.8
	5	28.6	24.5	26.6
	8	37.9	31.7	34.8
	10	41.4	35.3	38.4

[a] Applied August, 1928, as a spray at a rate of 484 pounds per acre.

TABLE 2.—*Effect of sodium chlorate upon the catalase activity of bindweed roots taken from the soil in foot sections from the first to the fourth, inclusive, April, 1929*

Treatment of plots	Oxygen per gram of roots, per unit of time in minutes, liberated by roots taken from soil at indicated depths											
	First foot			Second foot			Third foot			Fourth foot		
	2 min.	4 min.	6 min.	2 min.	4 min.	6 min.	2 min.	4 min.	6 min.	2 min.	4 min.	6 min.
	C. c.	*C. c.*	*C. c.*	*C. c.*	*C. c.*	*C. c.*	*C. c.*	*C. c.*	*C. c.*	*C. c.*	*C. c.*	*C. c.*
None	58.0	83.0	98.0	26.0	41.0	50.0	19.3	29.3	36.4	21.0	32.3	40.3
Sodium chlorate [a]	17.6	26.0	32.0	9.4	16.0	20.6	8.8	14.1	16.1	17.0	27.5	34.8

[a] Applied August, 1928, as a spray at a rate of 484 pounds per acre.

About a month after these determinations were made root samples were obtained from two additional plots from another area. One of these plots had received a 484-pound-per-acre application of sodium chlorate in August of the previous year; the other had not been treated. In the untreated plot the catalase activity of the roots of the first foot section was much higher than that of the second, which in turn was higher than that of the third and fourth. (Table 2.) This is more easily seen in Figure 2 which gives only the 4 minute values from Table 2. The chlorate treatment distinctly lowered the activity of the roots of the first foot section but had less effect on the activity of those of the second and third foot sections. The activity of the roots of the fourth foot section was about the same on both treated and untreated plots (fig. 2), indicating that there was a progressive decrease in the toxicity of the chlorates as far as the fourth foot area.

An examination of the plots referred to in Figures 1 and 2 during the following season, or two years after the chlorate treatment, dis-

closed practically no surface growth of the bindweed, thus showing that the roots had been injured to such extent that they died.

In June, 1930, catalase determinations were made on the roots from plots that had been treated in July, 1929. In the second and third foot sections catalase activity was higher in these roots (Table 3) than in roots from untreated plots, pointing to the possibility that a toxicity had been produced sufficient to stimulate but not to destroy the roots. The catalase activity of the roots from a plot that

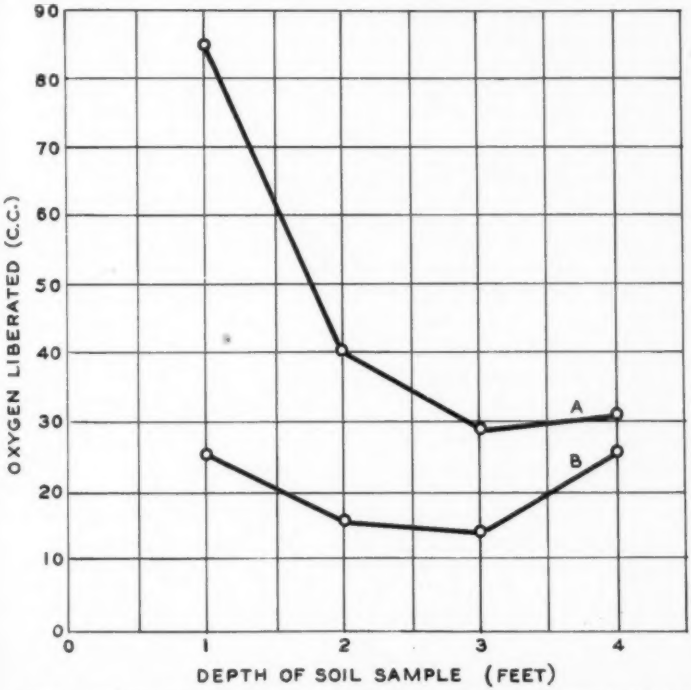

FIGURE 2.—Catalase activity of normal (A) bindweed roots and of roots from bindweed which had been sprayed with sodium chlorate (B); samples taken from 1 to 4 feet below the soil surface. This figure is based upon the 4-minute values given in Table 2

had received a similar chemical treatment in September instead of July was about the same as that of untreated roots. A large amount of surface growth appeared on these plots during the year after the treatment. The spray that was used consisted of a solution that contained 21 per cent of sodium chlorate and 45 per cent of magnesium chloride applied at the rate of 138 pounds per acre. This was one of the weakest of a series of chemical treatments that was under study and was selected for catalase determinations for that reason.

TABLE 3.—*Effect of chemical treatment of plots upon the catalase activity of bindweed roots taken from the first to the third foot of soil from the surface in June, 1930*

Treatment of plots	Oxygen per gram of roots, liberated in 4 minutes by roots taken from soil at indicated depths		
	First foot	Second foot	Third foot
	C. c.	*C. c.*	*C. c.*
None	34.1	16.3	11.2
$NaClO_3$-$MgCl_2$ mixture [a]	29.3	27.7	29.1
$NaClO_3$-$MgCl_2$ mixtures [b]	30.7	15.8	18.6
$NaClO_3$-$CaCl_2$ mixture [a]	11.8	28.0	28.8

[a] Applied in July, 1929. [b] Applied in September, 1929.

Another plot that was sampled had been sprayed with a sodium chlorate-calcium chloride mixture, 60.7 and 33 per cent of each, respectively, at the rate of 300 pounds per acre. Catalase activity (Table 3) of the roots thus treated was somewhat reduced in the first and increased in the second and third foot sections. Only a partial eradication of the weed was obtained on this plot, and this was forecast by an insufficient reduction in catalase activity as recorded above.

DISCUSSION

When a treatment with chlorates causes the catalase activity of bindweed roots to be distinctly lowered, as shown in Figures 1 and 2, it may be expected that the plant will eventually die. This indicates that the catalase activity of the roots of a poisoned plant is a good index of its reserve vitality. It is probable also that the chlorate ion inhibits the other enzymatic activities of a plant in a similar way. In certain tests where sodium chlorate was added to catalase-active mixtures there was but little effect upon catalase activity at first, but the toxic effects increased after the mixtures had stood awhile. This is in conformity with the general knowledge that when the aerial portions of plants are sprayed with chlorates they die more slowly than when sprayed with other materials such as arsenic compounds. It is possible that the more slowly acting but higher toxicity of chlorates is due to a gradual decomposition within the plant tissues accompanied by a liberation of nascent oxygen.

Tables 2 and 3 and Figure 2 show that the physiological action of chlorates, as indicated by catalase values, becomes progressively less pronounced in the lower root levels and in the fourth foot section is practically without effect. These observations were made in the spring and early summer on the roots of plants sprayed late in the previous summer. The climate of eastern Washington, with its numerous fall rains and frozen-soil conditions during the winter, precluded the taking of sampling during those periods. If, as suggested above, the toxic action of chlorates is due to a liberation of nascent oxygen, this action would be slower during the colder winter months and would thus permit a more thorough penetration of the salt before its decomposition, leaving a comparatively harmless residue of chloride salts. This may be the explanation for the general

belief that chlorates are most effective as weed killers when sprayed upon fairly mature plants; that is, at the beginning of cooler weather.

SUMMARY

An apparatus for determining the catalase activity of plants is described, and a method for preparing the root samples is given.

It was found that the catalase activity of roots of the bindweed (*Convolvulus arvensis*) was greatly decreased after the plants had been thoroughly sprayed with chlorates.

In one plot treated with sodium chlorate in August, the calatase activity of roots obtained the following March from the surface foot was found to be one-half that of untreated roots.

In another plot receiving similar treatment the catalase activity of roots in the first, second, third, and fourth foot sections was respectively one-fourth, one-half, one-half, and five-sixths that of roots from similar sections of an untreated plot.

In plots receiving a much lighter application of chlorate the catalase activity of the roots was reduced little or not at all in the first foot and not at all in the second foot section. An inspection of surface growth on these plots at a later date showed that only partial eradication had been obtained.

It appears that the toxicity of the chemical must be sufficient to distinctly lower the catalase activity of the roots to a depth of 2 feet if complete eradication of the bindweed is to be obtained.

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